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(57) Abstract

We have discovered *p40*, the shortest variant of a new human *p53* homologue (*p40/p51/p63/p73H*). We have also found that it plays a role in cancer. Low level amplification of the *p40* locus accompanied by RNA and protein overexpression was observed in primary lung cancers, and head and neck cancer cell lines. *P40* protein overexpression in primary lung tumors was limited to squamous cell carcinoma, tumors known to harbor a high frequency of *p53* mutations. Overexpression of *p40* in Rat 1a cells led to an increase in soft agar growth and tumor size in mice. We searched for *p40* binding proteins using the yeast two–hybrid system. *P53* was the most common binding target of the 1.6×10^6 clones screened from a mouse embryonic library. Moreover, coexpression of *p40* and *p53* led to a decrease in *p53* transcriptional activity. Our results support the notion that *p40* plays an oncogenic role in human cancer.

P40 PROTEIN ACTS AS AN ONCOGENE

This application claims the benefit of provisional application Serial No. 60/079,736 filed March 27, 1998, the disclosure of which is expressly incorporated herein.

5   The U.S. government retains certain rights in the invention due to its support via grant no. CA588401 from the National Cancer Institute.

TECHNICAL FIELD OF THE INVENTION

This invention is related to the field of cancer diagnostics and therapeutics.

10   ## BACKGROUND OF THE INVENTION

p53 is the most commonly inactivated gene in human cancer and loss of critical *p53* pathways are central to tumorigenesis[1,2]. The *p53* protein binds specific DNA sequences and transcriptionally activates responsive genes. *p53* gene mutations that occur in human cancer produce abnormal *p53* proteins that

15   are unable to bind DNA and promote the transcription and modulation of its target genes[3]. Furthermore, mutant *p53* protein can act in a dominant negative manner by disturbing the function of wild type *p53* protein and its ability to regulate cell proliferation[4]. *p53* mutant protein also heterodimerizes with wild type *p53* and results in a conformational change of the protein that no longer

20   binds to *p53* regulating cis-elements[4,7].

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There is a continuing need in the art for a more complete understanding of the components of the pathways in which p53 acts. Moreover, there is a continuing need for improved diagnostic and therapeutic methods for treating cancers.

5   ## SUMMARY OF THE INVENTION

It is an object of the present invention to provide an isolated and purified protein useful for diagnosis and classification of cancers.

It is another object of the present invention to provide a fusion protein useful for raising antibodies and drug screening.

10   It is an object of the present invention to provide a preparation of antibodies useful for therapeutic intervention in cancer.

Another object of the invention is to provide a cDNA molecule, a subgenomic polynucleotide, and a nucleic acid construct which encode a protein useful for diagnosis and classification of cancers.

15   Another object of the invention is to provide methods of diagnosing and classifying neoplastic tissues of humans.

Another object of the invention is to provide methods of screening test compounds useful for treating cancers.

Another object of the invention is to provide a cell useful for screening test compounds useful for treating cancers.

20   It is yet another object of the invention to provide a method for visualizing a human chromosomal arm 3q.

It is still another object of the invention to provide therapeutic compositions and methods for treating neoplasia.

25   These and other objects of the invention are achieved by one or more of the following embodiments. In one embodiment an isolated and purified p40 protein is provided. It has an amino acid sequence which is at least 99% identical to SEQ ID NO:2. Percent identity is determined using a Smith-Waterman homology search algorithm using an affine gap search with a gap

30   open penalty of 12 and a gap extension penalty of 1.

According to another embodiment of the invention a p40 fusion protein is provided which comprises a first protein segment and a second protein segment fused together by means of a peptide bond. The first protein segment consists of a p40 protein as shown in SEQ ID NO:2.

5   According to yet another embodiment of the invention a preparation of antibodies is provided. The antibodies specifically bind to a p40 protein having an amino acid sequence as shown in SEQ ID NO:2. The antibodies do not bind to p53 as shown in SEQ ID NO: 4.

          In still another embodiment of the invention a cDNA molecule is

10   provided which encodes a p40 protein having an amino acid sequence which is at least 99% identical to SEQ ID NO:2. Another aspect of the invention is a cDNA molecule which is at least 99% identical to the nucleotide sequence shown in SEQ ID NO:1. In still another aspect of the invention a nucleic acid construct is provided. The construct comprises: a promoter and a

15   polynucleotide segment encoding a p40 protein as shown in SEQ ID NO:2. The polynucleotide segment is located downstream from the promoter. Transcription of the polynucleotide segment initiates at the promoter and the promoter is not the endogenous p40 promoter. Also provided is a host cell which comprises such a nucleic acid construct.

20   According to another embodiment of the invention a method is provided for diagnosing and classifying a neoplastic tissue of a human. Amplification of a *p40* gene is detected in a tissue suspected of being neoplastic. The *p40* gene has the coding sequence shown in SEQ ID NO:1. Amplification indicates neoplasia of the tissue.

25   Also provided is another method of identifying or classifying a neoplastic tissue of a human. Expression of a first *p40* gene in a first tissue of a human suspected of being neoplastic is compared with expression of a second *p40* gene in a second tissue of the human which is normal. Increased expression of the first *p40* gene relative to the second *p40* gene identifies the

30   first tissue as being neoplastic and having a *p40* amplification.

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According to another aspect of the invention a method is provided of screening test compounds for the ability to modulate the binding of a p40 protein to a p53 protein. A test compound is contacted with a first protein comprising a p53 protein as shown in SEQ ID NO:4 and a second protein

5   comprising a p40 protein as shown in SEQ ID NO:2. The first and second proteins bind to each other in the absence of the test compound. The amount of the first protein which is bound or unbound to the second protein or the amount of the second protein which is bound or unbound to the first protein in the presence of the test compound is determined. A test compound which

10   modulates the amount of bound first or second protein or which modulates the amount of unbound first or second protein is a potential drug for treating cancer.

Another aspect of the invention is a method of screening test compounds for the ability to modulate the binding of a p53 protein to a p40

15   protein. A cell is contacted with a test compound. The cell comprises (i) a first fusion protein comprising a p40 protein as shown in SEQ ID NO:2 and either a DNA binding domain or a transcriptional activating domain; (ii) a second fusion protein comprising a p53 protein as shown in SEQ ID NO:4 and either a DNA binding domain or a transcriptional activating domain; and (iii)

20   a reporter gene comprising a DNA sequence to which the DNA binding domain specifically binds. If the first fusion protein comprises a DNA binding domain, then the second fusion protein comprises a transcriptional activating domain, and if the first fusion protein comprises a transcriptional activating domain, then the second fusion protein comprises a DNA binding domain. The

25   interaction of the first and second fusion proteins reconstitutes a sequence-specific transcription activating factor. The expression of the reporter gene is measured. A test compound which modulates the expression of the reporter gene is a potential anti-cancer drug.

Also provided by the present invention is a cell which comprises three

30   recombinant DNA constructs: a first construct encodes a first polypeptide fused to a sequence-specific DNA-binding domain; a second construct encodes

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a second polypeptide fused to a transcriptional activation domain; and a third construct comprises a reporter gene downstream from a DNA element which is recognized by the sequence-specific DNA-binding domain. Either the first polypeptide comprises a p40 protein as shown in SEQ ID NO:2 and the second

5   polypeptide comprises a p53 protein as shown in SEQ ID NO:4, or the first polypeptide comprises a p53 protein as shown in SEQ ID NO:4 and the second polypeptide comprises a p40 protein as shown in SEQ ID NO:2.

Another embodiment provided by the present invention is a method of visualizing a human chromosomal arm 3q. A preparation of metaphase human

10   chromosomes is contacted with a nucleotide probe comprising at least 12 contiguous nucleotides selected from the nucleotide sequence shown in SEQ ID NO:1. A chromosome which specifically hybridizes to the nucleotide probe is detected and identified as a human chromosomal arm 3q.

According to another aspect of the invention a therapeutic composition

15   is provided for treating neoplasia. The composition comprises a therapeutically effective amount of an antisense *p40* polynucleotide and a pharmaceutically acceptable carrier.

According to another aspect of the invention a therapeutic composition is provided for treating neoplasia. The therapeutic composition comprises a

20   therapeutically effective amount of an antibody which specifically binds to a human p40 protein and a pharmaceutically acceptable carrier.

Yet another aspect of the invention is a method of treating neoplasia. A therapeutically effective amount of a therapeutic *p40* composition is administered to a patient with neoplasia, whereby the patient's neoplasia is

25   reduced.

The present invention thus provides the art with an important new drug target which is important in the process of cancer development and progression. The target can be used diagnostically and therapeutically as well as in the screening and testing for new pharmacologic agents.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 Detection of *p40* expression by Northern analysis. Figure 1a, *p40* transcripts in head and neck squamous cell carcinoma (HNSCC) cell lines and a sarcoma cell line, SaOs2. *p40* expression is observed in all HNSCC cell lines

5 in contrast to a lack of *p40* expression in the SaOs2 cell line. A human β-actin probe was used as an internal control. Figure 1b, Ten μg of total RNA extracted from tumor (T) and normal (N) tissues of 5 different patients with primary lung cancers were hybridized with a ^{32}P-labeled probe for *p40* and β-actin. *p40* expression varying intensity is observed in tumor RNA of cases

10 L2, L10, L12, but is absent in all normal tissue controls.

Figure 2 Detection of *p40* gene amplification by FISH analysis. Figure 2a, Low level *p40* gene amplification (BAC probe, red) was observed on metaphase and interphase nuclei of the HNSCC cell line (FADU) compared to a chromosome 3 centromeric probe (green). FADU is known to have an

15 abnormal chromosome 3 karyotype; der(3)t(3:8)(q21:q?). Six signals from the BAC probe were seen on the telomeric end of the long arm of chromosome 3 and on the short arm of chromosome 3 compared to 4 signals from the chromosome 3 centromeric probe. As a control, normal lymphocytes were subjected to the same FISH analysis to confirm that the *p40* probe had no

20 cross-hybridization with other chromosomal regions. Figure 2b, A similar FISH analysis on a primary squamous cell lung carcinoma (T21) demonstrating 2 centromeric signals (green) and 3-4 *p40* signals (red) per tumor cell.

Figure 3 *p40* immunoreactivity in squamous cell carcinoma of the lung (T1). Nests of infiltrating tumor cells demonstrate intense nuclear staining (arrow)

25 after incubation with a polyclonal *p40* antibody with absence of expression in surrounding normal cells. This tumor had genomic amplification of *p40* and a *p53* mutation (Table 2).

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Figure 4 Tumor growth in nude mice. Rat 1a-*p40* cells developed into significantly larger tumors compared with Rat 1a-no cells at day 21 (p=0.0335, t-test), day 24 (p=0.0445), and day 31 (p=0.0009). Each curve represents the average volume of 5 tumors as shown (Methods). On day 31, Rat 1a-*p40*

5   tumors averaged 184.2±49.6 mm^3 while Rat 1a-no tumors averaged 47.2±33.1 mm^3 in size.

Figure 5 Two-hybrid yeast expression analysis of interaction of *p40* with *p53*. *Saccharomyces cerevisiae* (strain SFY526) were co-transformed by the lithium acetate method with pGal4-BD-p40 (alone), pGal4-AD-p53-1 (alone),

10   pGal4-AD-p53-2 (alone) or with various combinations of pGal4-BD-p40 and pGal4-AD-p53-1, or pGal4-AD-p53-2, or pGal4-AD. As controls, yeast were co-transformed with control plasmids (pGal4-BD-p53 with pGal4-AD-SV40, or pGal4-BD-lamin C with pGal4-AD-SV40). First, transformed yeast were selected on double drop-out agar plates (Trp-/Leu-). Then colonies obtained

15   from each group were re-streaked on new triple drop-out agar plates (Trp-/Leu-/His-). After 3 day incubation final plates were photographed demonstrating growth of yeast with the two p53+p40 clones and the control p53+SV40 clone.

Figure 6a shows a comparison of p40 to other p53 homologues. The

20   highlighted segments are the transcriptional activation domain, the DNA binding domain, and the oligomerization domain. **Figure 6b** shows a multiple tissue northern blot probed with a cloned 180 bp product of *p40* and *GAPDH* as a control. *Lanes:* 1, spleen; 2, thymus; 3, prostate; 4, testis, 5, ovary; 6, small intestine; 7, colon; 8, peripheral blood leukocytes. **Figure 6c** shows a

25   FISH analysis of a BAC8836 probe (Genome Systems) on a metaphase nucleus of a normal human lymphocyte with the centromeric probe of chromosome 3 hybridized simultaneously. Signals from the BAC probe were seen on the telomeric end of the long arm of chromosome 3.

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Figure 7 shows *in vitro* binding pull-down analysis of p40/p53 complexes. **Figure 7a** shows immunoblotting of a pure GST-p53 fusion protein (10 ng) with a monoclonal antibody to p53 (BP53-12, raised against amino terminal residues 20-25, dilution 1:1000) and immunoblotting of a pure

5   GST-midkine (20 ng) with a polyclonal antibody to midkine (M-18, raised against carboxyl terminal residues 122-140, dilution 1:1000). **Figure 7b** shows cell-free translation of pCR2.1-p40 (20% input, lane 1); pull-down assay of labeled p40 protein with GST-p53 protein (100 ng) followed by precipitation with glutathione-agarose beads (lane 2); pull-down assays of the labeled p40

10   protein with GST-midkine (100 ng) followed by precipitation with glutathione-agarose beads (lane 4). Labeled precipitates were resolved by denaturing gel electrophoresis and dried gels were autoradiographed. Immunoblots were visualized with secondary antibodies coupled to horseradish peroxidase followed by enhanced chemiluminescence.

15   **Figure 8a.** Saos-2 cells were infected with empty adenovirus (MOI=1, lane 1) or with P40 adenovirus (MOI=1, lane 2) for 1 h. **Figure 8b.** Cell lysates were incubated with glutathione-agarose beads (lane 1) or with a pure GST-midkine (lane 2) or with a pure GST-p53 (lane 3) for 2 h at 4° C. Mixes were precipitated with 50 μl (1:1) glutathione-agarose beads and washed.

20   Bound proteins were eluted with 10 mM reduced glutathione. Proteins were resolved by SDS-PAGE and visualized with a polyclonal antibody to p40 protein. For immunoprecipitation, cell lysates were precleared with a pre-immune rabbit serum (10 μg/500 μl) followed by a precipitation with goat anti-rabbit immunoglobulin-coupled agarose beads. Supernatants (Ad-p40, lane 4,

25   Ad4, lane 5) were incubated with a polyclonal antibody to p40 (5 μg) for 16 h at 4° C followed by precipitation with goat anti-rabbit immunoglobulin-coupled agarose beads for 1 h at room temperature, and analyzed by immunoblotting with a monoclonal antibody to p53 (amino terminal).

       Figure 9 shows a schematic comparison of members of the p53 family

30   including p40 splice variants.

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DETAILED DESCRIPTION

We have isolated a new human *p53* homologue, *p40*, and localized the gene to the distal long arm of chromosome 3[8]. The DNA binding domain and the oligomerization domain of *p40* display strong conservation of amino acid

5   residues with *p53*, raising the possibility that human *p40* may also bind key *p53* DNA binding sites in the human genome and/or interact with *p53*. Concurrently, an alternative *p40* transcript termed *p51*, was cloned and shown to suppress colony formation in cell lines and to transcriptionally activate *p21* in a fashion similar to the *p53* tumor suppressor gene[9]. Subsequently, another

10   group of splice variants (*p63*) were also described[10]. A transcript that lacked the N-terminal transactivation domain of *p53* (ΔN*p63*) was found to act in a dominant negative fashion and to be able to suppress *p53* transactivation. In contrast to these other variants, we found no evidence of a tumor suppressor function for *p40*. Instead, we observed *p40* gene overexpression in head and

15   neck cancer cell lines and primary lung cancers associated with a low level increase of *p40* copy number. In transformation assays, Rat 1a cells with *p40* expression developed larger colonies in soft agar and bigger tumors in nude mice compared to cells with an empty vector. Interestingly, binding to *p53* was identified through a yeast two-hybrid system in which *p40* was used as the bait.

20   As shown previously for ΔN*p63*, coexpression of *p40* and *p53* led to a reduction of *p53* transcriptional activity. Our data suggest that *p40* complexes with *p53* and diminishes its transcriptional activity, supporting the notion that *p40* may play an oncogenic role in certain cancers.

The *p40* gene maps to human chromosomal arm 3q, a region known for

25   aberrations including deletions and amplifications in human cancers, such as bladder cancer. Amplifications of this region of chromosomal arm 3q have been observed in squamous cell carcinomas. Such carcinomas occur in head, neck, cervix, skin and lung.

Despite the initial enthusiasm surrounding the cloning of a family of

30   *p53* homologues, there has been little evidence to date as to the role of these genes in the development of human cancers. Abnormal expression of *p73* has

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been seen in certain cancers but has been disputed by others[11,12]. Although other splice variants with a TA domain have been shown to be growth suppressive[9], our results do not support a tumor suppressor role for *p40* in head and neck and lung cancers.

Conversely, our work provides tantalizing evidence that *p40* may have an oncogenic role in human cancer based on the following observations: 1) the *p40* gene is amplified in primary lung cancers and HNSCC cell lines by FISH analysis; 2) chromosomal amplification is associated with increased expression of RNA by Northern analysis; 3) increased gene expression is associated with increased protein accumulation by immunohistochemistry in squamous cell cancer; 4) increased *p40* expression in Rat 1a cells leads to a transformed phenotype; and 5) *p40* interacts with *p53* and suppresses its transactivation activity on target genes.

Our data for low level *p40* amplification are consistent with recent reports indicating the presence of amplification of chromosomal arm 3q in squamous cell lung carcinoma. Comparative genomic hybridization (CGH) studies indicate that squamous cell lung carcinoma commonly displays overrepresentation of the distal arm of Chromosomal arm 3q[13]. In one study, two candidate genes, BCHE and SLC2A2, were identified as possible oncogenes due to overexpression in 40% of squamous cell lung carcinomas[14]. We have demonstrated consistent *p40* overexpression at the RNA and protein level in those squamous cell carcinomas with an increase in *p40* copy number. This combined evidence strongly suggests a role for *p40* overexpression in the progression of these cancers.

Further support for the role of *p40* as an oncogene comes from our functional assays. *P40* overexpression in Rat 1a cells led to a significant increase in the number and size of colonies in soft agar consistent with previous results in *bonafide* oncogenes[15,16]. The ability of these overexpressing cells to form larger tumors in nude mice supports the notion that *p40* overexpression provides a growth advantage to tumor cells *in vivo*.

The physical interaction of *p53* and *p40* based on the yeast two-hybrid system and immunoprecipitation studies suggest an intimate association between the two members of the *p53* family. Of all possible protein targets in an unbiased test, *p53* was the most common clone identified with *p40* as the

5   bait. *p53* protein homodimerizes and forms a tetramer before binding to DNA and transactivating downstream targets[17]. The cloning of new *p53* homologues now suggests a more complex pathway for *p53* action on downstream genes. It is quite plausible that *p40*, and perhaps other members of the *p53* family of genes, may bind as heterodimers in different complexes. Depending on the

10   individual components of these heterodimers, the protein complexes might be activating for certain downstream genes or, as suggested by co-transfection studies here and previously, might lead to a decrease in *p53* transactivation. One recent study demonstrated no evidence of interaction between all *p73* splice variants and *p53* suggesting that the interaction between *p40* and *p53*

15   described here may be more unique[18]. We still do not know the exact interaction motif between the 2 proteins and whether *p40* or *p53* is the critical gene that is regulated by this interaction. Further co-immunoprecipitation studies will add further light to the nature of the interaction between *p40* and *p53*.

20   As noted above, *p73* and some splice variants of *p40* contain a putative transactivating domain capable of inducing apoptosis and tumor suppression, but there has been little evidence of inactivating point mutations or other evidence of gene inactivation in human tumors[9,10,19,20]. It appears that ΔN*p63* and *p40*, lacking the acidic N-terminal domain, can act in a dominant negative

25   fashion to diminish transactivation by *p53*. For *p73*, it was recently shown that different splice variants may have quite distinct effects on various *p53* cis-elements[21]. Similar studies should shed further light on the specificity of *p40* splice variants on target *p53* promoters and their activity in the presence of *p53*.

30   Finally, our data raise a question as to why squamous cell tumors with *p53* mutations appear to be associated with an increase in *p40* expression. At first

glance, this intriguing association suggests that *p40* and *p53* may be targeting different parallel pathways in tumorigenesis. However, the binding of *p40* and *p53* demonstrated by the yeast two-hybrid system suggests convergence of these two proteins in an oncogenic pathway. It is possible that *p40* expression

5   diminishes *p53* activity providing some growth advantage to the cell followed by the eventual emergence of a *p53* mutation and complete abrogation of *p53* function. On the other hand, one could hypothesize that *p53* mutation occurs first and that mutant *p53* protein does not bind to *p40* leading to an accumulation of *p40* and possible promotion of its oncogenic function. As

10 shown previously, *p53* mutation can lead to an increase in genomic plasticity associated with polysomy and gene specific amplification[22,23]. This might explain the association between *p53* mutation and low level chromosomal arm 3q amplification in human tumors.

The evidence presented here supports the notion that *p40* amplification

15 and overexpression plays a role in the development of squamous cell carcinoma of the upper aerodigestive tract.

Human p40 mRNA comprises a 5 kb transcript. Northern blots of human polyA[+] RNA probed with a *p40* nucleotide probe demonstrate that the 5 kb transcript is expressed, *inter alia*, in prostate.

20 Human p40 polypeptides preferably comprise at least 6, 10, 12, 14, 15, 18, 20, 25, 30, or 35 contiguous amino acids of the amino acid sequence shown in SEQ ID NO:2. Human p40 proteins and polypeptides can be isolated and purified from human cells such as prostate cells.

Human p40 protein has the amino acid sequence shown in SEQ ID

25 NO:2. Any naturally occurring variants of this sequence which may occur in human tissues and which have, for example, oncogenic or proliferation-inducing activity, are within the scope of this invention. Nonnaturally occurring p40 variants which differ by as much as 1 % are also encompassed.

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Preferably the amino acid changes in p40 variants or derivatives are conservative amino acid changes, *i.e.*, substitutions of similarly charged or uncharged amino acids. A conservative amino acid change involves substitution of one of a family of amino acids which are related in their side chains. Naturally occurring amino acids are generally divided into four families: acidic (aspartate, glutamate), basic (lysine, arginine, histidine), non-polar (alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), and uncharged polar (glycine, asparagine, glutamine, cystine, serine, threonine, tyrosine) amino acids. Phenylalanine, tryptophan, and tyrosine are sometimes classified jointly as aromatic amino acids. It is reasonable to expect that an isolated replacement of a leucine with an isoleucine or valine, an aspartate with a glutamate, a threonine with a serine, or a similar replacement of an amino acid with a structurally related amino acid will not have a major effect on the binding properties of the resulting molecule, especially if the replacement does not involve an amino acid at a binding site involved in an interaction of p40 protein. Whether an amino acid change results in a functional p40 protein or polypeptide can readily be determined by assaying the properties of the protein or polypeptide, as described below. Variants of p40 proteins have substantially the same biological activities, that is, for example, p53-binding activities which are of the same type as a p40 protein having the amino acid sequence shown in SEQ ID NO:2, although the activities may differ in degree.

p40 proteins or polypeptides can be purified by any method known in the art. These methods include, but are not limited to, size exclusion chromatography, ammonium sulfate fractionation, ion exchange chromatography, affinity chromatography, crystallization, electrofocusing, immunoprecipitation, immunoaffinity chromatography, and preparative gel electrophoresis. The skilled artisan can readily select methods which will result in a preparation of p40 protein or polypeptide which is substantially free from other proteins and from carbohydrates, lipids, or subcellular organelles. A preparation of isolated and purified p40 protein is at least 80% pure;

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preferably, the preparations are 90%, 95%, or 99% pure. Purity of the preparations may be assessed by any means known in the art, such as SDS-polyacrylamide gel electrophoresis.

p40 proteins and polypeptides can also be produced by recombinant DNA methods or by synthetic chemical methods. For production of recombinant p40 proteins or polypeptides, coding sequences selected from the nucleotide sequence shown in SEQ ID NO:1 can be expressed in known prokaryotic or eukaryotic expression systems. Bacterial, yeast, insect, or mammalian expression systems can be used, as is known in the art. Alternatively, synthetic chemical methods, such as solid phase peptide synthesis, can be used to synthesize p40 protein or polypeptides.

p40 fusion proteins are useful for generating antibodies against p40 amino acid sequences and for use in various assay systems. For example, p40 fusion proteins can be used to identify proteins which interact with p40 protein and influence its function. Physical methods, such as protein affinity chromatography, or library-based assays for protein-protein interactions, such as the yeast two-hybrid or phage display systems, can be used for this purpose. Such methods are well known in the art and can be used, *inter alia*, as drug screens.

A p40 fusion protein comprises two protein segments fused together by means of a peptide bond. The first protein segment comprises at least 8, 10, 12, 15, or 20 contiguous amino acids of a p40 protein. The amino acids can be selected from the amino acid sequence shown in SEQ ID NO:2 or from a naturally or nonnaturally occurring biologically active variant of that sequence, such as those described above. The first protein segment can also be a full-length p40 protein. The second protein segment can be a full-length protein or a protein fragment or polypeptide. The fusion protein can be labeled with a detectable marker, as is known in the art, such as a radioactive, fluorescent, chemiluminescent, or biotinylated marker. The second protein segment can be an enzyme which will generate a detectable product, such as β-galactosidase or other enzymes which are known in the art. The second protein can have any

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useful property, such as affinity to an analytic reagent or immunogenicity. The first protein segment may be N-terminal or C-terminal to the second protein segment, as is convenient.

Techniques for making fusion proteins, either recombinantly or by covalently linking two protein segments, are also well known. Recombinant DNA methods can be used to construct p40 fusion proteins, for example, by making a DNA construct which comprises coding sequences selected from SEQ ID NO:1 in proper reading frame with nucleotides encoding the second protein segment and expressing the DNA construct in a host cell, as described below.

Isolated and purified p40 proteins, polypeptides, or fusion proteins can be used as immunogens, to obtain a preparation of antibodies which specifically bind to a p40 protein. The antibodies can be used to detect p40 proteins in human tissue and fractions thereof. The antibodies can also be used to detect the presence of amplification of the p40 gene which results in overexpression of the p40 protein or in expression.

p40-specific antibodies specifically bind to a p40 polypeptide. p40-specific antibodies bind to p40 and have a measurably higher binding affinity for a p40 polypeptide than for non-p40 polypeptides, particularly p53. Higher affinity by a factor of 5, or 10 is preferred, more preferably by a factor of 100. The antibodies may be polyclonal or monoclonal. They are preferably raised against portions of the protein which differ significantly from p53 and its other homologues. See Figure 6 which compares the amino acid seqeunce of p40 to other p53 homologues.

Preparations of polyclonal and monoclonal p40 antibodies can be made using standard methods known in the art. The antibodies specifically bind to epitopes present in p40 proteins having the amino acid sequence shown in SEQ ID NO:2 or in naturally or non-naturally occurring variants of that sequence. Preferably, the p40 epitopes are not present in other human proteins. Typically, at least 6, 8, 10, or 12 contiguous amino acids are required to form an epitope. However, epitopes which involve non-contiguous amino acids may

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require more, *e.g.*, at least 15, 25, or 50 amino acids. Antibodies which specifically bind to p40 proteins provide a detection signal at least 5-, 10-, or 20-fold higher than a detection signal provided with other proteins when used in Western blots or other immunochemical assays. Preferably, antibodies which
5   specifically bind p40 proteins do not detect other proteins in immunochemical assays and can immunoprecipitate p40 proteins from solution.

Human p40 antibodies can be purified by methods well known in the art. Preferably, the antibodies are affinity purified, by passing antiserum over a column to which a p40 protein, polypeptide, or fusion protein is bound. The
10   bound antibodies can then be eluted from the column, for example, using a buffer with a high salt concentration.

Purified and isolated *p40* subgenomic polynucleotides can be used, *inter alia*, as primers to obtain additional copies of the polynucleotides, to express human p40 mRNA, protein, polypeptides, or fusion proteins, and as probes for
15   identifying *p40* coding sequences. The probes can also be used to identify the long arm of a human chromosome 3, as described below.

Purified and isolated *p40* subgenomic polynucleotides of the invention comprise at least 11, 13, 15, 18, 20, 25, or 30 contiguous nucleotides selected from SEQ ID NO:1. Subgenomic *p40* polynucleotides according to the
20   invention contain less than a whole chromosome.   Preferably,   the polynucleotides are intron-free, *i.e.*, cDNA.

Subgenomic *p40* polynucleotides can be isolated and purified free from other nucleotide sequences using standard nucleic acid purification techniques. For example, restriction enzymes and probes can be used to isolate
25   polynucleotide fragments which comprise p40 coding sequences. Isolated polynucleotides are in preparations which are free or at least 90% free of other molecules.

DNA fragments derived from a p40-encoding DNA sequence are also included herein. A DNA fragment derived from a p40 coding sequence has the
30   same or substantially the same basepair sequence as a region of the coding

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sequence of the entire *p40* molecule. Preferably the DNA fragment has at least 99% identity with *p40*.

Complementary DNA encoding p40 proteins can be made using reverse transcriptase, with *p40* mRNA as a template. The polymerase chain reaction

5   (PCR) can be used to obtain *p40* polynucleotides, using either human genomic DNA or cDNA as a template. Alternatively, synthetic chemistry techniques can be used to synthesize polynucleotide molecules of the invention. The degeneracy of the genetic code allows alternate nucleotide sequences to be synthesized which will encode a p40 protein having the amino acid sequence

10   shown in SEQ ID NO:2. All such nucleotide sequences are within the scope of the present invention.

A *p40* subgenomic polynucleotide of the present invention can be used in an expression construct, to express all or a portion of a p40 protein in a host cell. The host cell comprising the expression construct can be prokaryotic or

15   eukaryotic. A variety of host cells for use in bacterial, yeast, insect, and human expression systems are available and can be used to express the expression construct. The expression constructs can be introduced into the host cells using any technique known in the art. These techniques include transferrin-polycation-mediated DNA transfer, transfection with naked or encapsulated

20   nucleic acids, liposome-mediated cellular fusion, intracellular transportation of DNA-coated latex beads, protoplast fusion, viral infection, electroporation, and calcium phosphate-mediated transfection.

The expression construct comprises a promoter which is not the p40 endogenous promoter and which is functional in the particular host cell

25   selected. The skilled artisan can readily select an appropriate promoter from the large number of cell type-specific promoters known and used in the art. The expression construct can also contain a transcription terminator which is functional in the host cell. The expression construct comprises a polynucleotide segment which encodes all or a portion of a p40 protein. The

30   polynucleotide segment is located downstream from the promoter. Transcription of the polynucleotide segment initiates at the promoter. The

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expression construct can be linear or circular and can contain sequences, if desired, for autonomous replication.

The *p40* gene maps to human chromosome region 3q. Thus, the subgenomic polynucleotides of the invention can be used to identify this chromosome region in metaphase spreads of human chromosomes. Preparations of human metaphase chromosomes can be prepared using standard cytogenetic techniques from human primary tissues or cell lines. Nucleotide probes comprising at least 12 contiguous nucleotides selected from the nucleotide sequence shown in SEQ ID NO:1 are used to identify the human chromosome. The nucleotide probes can be labeled, for example, with a radioactive, fluorescent, biotinylated, or chemiluminescent label, and detected by well known methods appropriate for the particular label selected. Protocols for hybridizing nucleotide probes to preparations of metaphase chromosomes are well known in the art. A nucleotide probe will hybridize specifically to nucleotide sequences in the chromosome preparations which are complementary to the nucleotide sequence of the probe. A probe which hybridizes specifically to the *p40* gene does not hybridize to nucleotide sequences present in other human genes. A probe which hybridizes specifically to a p40 gene provides a detection signal at least 5-, 10-, or 20-fold higher than the background hybridization provided with non-*p40* coding sequences.

A human chromosome which specifically hybridizes to a p40 nucleotide probe is identified as a human chromosome 3. In particular, the nucleotide probe identifies the long arm of human chromosome 3. Quantitation of the hybridization can be used to detect amplification of the p40 gene. Typically the amplification is less than 10-fold, and more typically it is less than 5-fold.

The present invention also provides a method to identify and classify neoplastic tissue in a human. The expression of a *p40* gene can be compared between a first tissue which is suspected of being neoplastic and a second tissue of the human which is normal. The normal tissue can be any tissue of the human, especially those which express the *p40* gene, including, but not limited to, prostate. The tissue suspected of being neoplastic can be derived from a

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different tissue type of the human, but preferably it is derived from the same tissue type. A difference in abundance of the *p40* gene, mRNA, or protein in the two tissues which are compared indicates a somatic mutation in the *p40* gene in the tissue of the human which was suspected of being neoplastic.

5   Alternatively, *p40* mRNA in the two tissues can be compared. PolyA$^+$ RNA can be isolated from the two tissues as is known in the art. For example, one of skill in the art can readily determine differences in the amount of *p40* mRNA transcripts between the two tissues that are compared, using Northern blots and nucleotide probes selected from the nucleotide sequence shown in

10   SEQ ID NO:1. Increased expression of *p40* mRNA in a tissue sample suspected of being neoplastic compared with the expression of *p40* mRNA in a normal tissue is indicative of neoplasia.

Any method for analyzing proteins can be used to compare two p40 proteins from matched samples. For example, antibodies of the present

15   invention can be used to detect p40 proteins in Western blots of protein extracts from the two tissues to detect changes in expression levels. A higher p40 protein expression level in a tissue suspected of being neoplastic compared with the p40 protein expression level in a normal tissue is indicative of neoplasia.

20   Similarly, comparison of *p40* gene sequences between a tissue of a human which is suspected of being neoplastic and a normal tissue of a human can be used to diagnose or classify cancers in the human. Observation of amplification of *p40* in the neoplastic tissue over time can be used to monitor the progression of the neoplasia in that tissue or to predict or monitor the

25   response of the neoplastic tissue to various therapeutic regimens.

According to another aspect of the invention, test compounds can be screened for utility as anti-cancer agents by the ability to suppress the expression or function of human p40 protein. Potential drugs can be contacted with cells and the expression of *p40* mRNA or protein monitored. This can be

30   accomplished by well known techniques in the art, such as Northern blots, immunoprecipitation, immunoblots, etc. Any technique which utilizes a *p40*

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nucleic acid probe or an antibody specific for p40 protein can be used. Other techniques, such as quantitative reverse PCR can also be employed.

In addition, *in vitro* techniques can be employed for testing the ability of candidate drugs to modulate p40 binding to p53. Such assays are well

5   within the skill of the art, once provided with the full sequences of the *p40* and *p53* genes and proteins. In addition, a yeast two-hybrid system can be used wherein one of the partners is p40 and one of the partners is p53. A cell which contains both of these partners can be contacted with test compounds and the augmentation or diminution of transactivation of the reporter gene can be

10   monitored.

Modulators of p53-p40 binding can be, for example, polypeptides, small peptides, peptoids, or other peptide analogs or other chemical inhibitors. Some of these inhibitors, such as related peptides or fusion proteins, can be developed rationally on the basis of knowledge of the sequences of p53 and

15   p40 which are disclosed herein. Alternatively, a random array of compounds can be screened for the ability to affect in a p53-p40 binding assay.

The test compounds can be pharmacologic agents already known in the art or can be compounds previously unknown to have any pharmacological activity. The compounds can be naturally occurring or designed in the

20   laboratory. They can be isolated from microorganisms, animals, or plants, and can be produced recombinantly, or synthesized by chemical methods known in the art.

A test compound can be contacted with a mixture of a p40 protein and a p53 protein. These molecules can be produced recombinantly or can be

25   synthesized using standard chemical methods. The proteins can be pre-bound prior to the step of contacting the test compound. Alternatively, the test compound can contact one of the proteins before the second protein is added.

The proteins can be in solution or one protein can be bound to a solid support. Alternatively, the proteins can be co-expressed in a cell. The proteins

30   can be unlabeled or labeled, for example, with a radioactive, fluorescent, or

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other detectable marker. They can be fusion proteins comprising p53 or p40 fused to another protein with or without a detectable enzymatic activity.

In one embodiment, the amount of at least one of the two proteins that is bound or unbound in the presence of the test compound is then measured. A number of methods can be used to measure the amount of bound or unbound protein. For example, the relative concentration of proteins bound to unbound can be detected by examining the apparent molecular masses of the molecules by size exclusion chromatography or by polyacrylamide gel electrophoresis under non-reducing conditions. If the proteins are in a cell, the transactivation by p53 of down-stream genes can be monitored as a means of measuring the amount bound. Other methods of measuring binding or dissociation of the proteins will readily occur to those of ordinary skill in the art and can be used. A test compound which modulates the quantity of one protein bound to a second protein is identified as a candidate therapeutic agent. While applicants are not bound by any particular theory of operation, enhanced binding of p40 could prevent its actions as an oncogene. Conversely, diminished binding of p53 could augment its actions as a tumor suppressor. According to the present invention a method is also provided of using the yeast two-hybrid technique to screen for test compounds which modulate with p53-p40 binding. The yeast two-hybrid technique is taught in Fields & Song, *Nature* 340, 245-46, 1989. In a preferred embodiment, a cell is contacted with a test compound. The cell comprises two fusion proteins, which can be supplied to the cell by means of recombinant DNA constructs. The first fusion protein comprises a DNA-binding domain. The second fusion protein comprises a transcriptional activating domain. The first fusion protein also comprises either (i) p40 or (ii) a p53. If the first fusion protein comprises p53, then the second fusion protein comprises p40. If the first fusion protein comprises p40, then the second fusion protein comprises p53. The cell also comprises a reporter gene comprising a DNA sequence downstream from a DNA element to which the first fusion protein binds.

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When the p40 and p53 proteins are bound together, the DNA binding domain and the transcriptional activating domain will be in close enough proximity to reconstitute a transcriptional activator capable of initiating transcription of the detectable reporter gene in the cell. The expression of the reporter gene in the presence of the test compound is then measured. A test compound that increases the expression of the reporter gene is a potential drug for increasing p53-p40 binding. A test compound that decreases the expression of the reporter gene is a potential drug for decreasing p53-p40 binding.

Many DNA binding domains and transcriptional activating domains can be used in this system, including the DNA binding domains of GAL4, LexA, and the human estrogen receptor paired with the acidic transcriptional activating domains of GAL4 or the herpes virus simplex protein VP16 (*see, e.g.*, Hannon *et al.*, *Genes Dev.* 7, 2378, 1993; A.S. Zervos *et al.*, *Cell* 72, 223, 1993; A.B. Votjet *et al.*, *Cell* 74, 205, 1993; Harper *et al.*, *Cell* 75, 805, 1993; B. Le Douarin *et al.*, *Nucl. Acids Res.* 23, 876, 1995). A number of plasmids known in the art can be constructed to contain the coding sequences for the fusion proteins using standard laboratory techniques for manipulating DNA (*see, e.g.*, Example 1, below).

Suitable detectable reporter genes include the *E. coli lacZ* gene, whose expression can be measured colorimetrically (see, *e.g.*, Fields and Song), and yeast selectable genes such as *HIS3* (Harper *et al.*; Votjet *et al.*; Hannon *et al.*) or *URA3* (Le Douarin *et al.*). Methods for transforming cells are also well known in the art. *See, e.g.*, a. Hinnen *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 75, 1929-1933, 1978. The test compound can comprise part of the cell culture medium or it may be added separately.

Antisense polynucleotides of the *p40* gene can be used to interfere with expression of the *p40* gene. Antisense polynucleotides are typically generated within the cell by expression from antisense constructs which contain the antisense *p40* strand as the transcribed strand from a promoter. A description of vectors which can be used to introduce antisense constructs to a cell is contained in U.S. Serial No. 08/869,309, which is expressly incorporated

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herein. Antisense *p40* polynucleotides will bind and/or interfere with the translation of *p40* mRNA.

The invention provides a therapeutic composition for inhibiting a p40 oncogene function in a cell. Inhibition of *p40* expression suppresses neoplasia,

5   dysplasia, or hyperplastic cell growth. The cell to be treated can be any cell of a human which expresses the *p40* oncogene, such as a cell of the prostate, lung, skin, head, neck, and cervix. Such cells include those in neoplasias of the tissues mentioned above as well as any other neoplastic cells which express the *p40* gene. The therapeutic composition can comprise the antisense strand of

10   all or a portion of human *p40* gene in a pharmaceutically acceptable carrier. The therapeutic compsoition can comprise an antisense construct which produceds an antisense strand RNA in a cell. The *p40* antisense product can be, *e.g.*, mRNA or DNA. Alternatively, the therapeutic composition can comprise antibodies which specifically bind to p40 proteins or polypeptides.

15   Pharmaceutically acceptable carriers are well known to those in the art. Such carriers include, but are not limited to, large, slowly metabolized macromolecules, such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, and inactive virus particles. Pharmaceutically acceptable salts can also be used in the

20   composition, for example, mineral salts such as hydrochlorides, hydrobromides, phosphates, or sulfates, as well as the salts of organic acids such as acetates, proprionates, malonates, or benzoates. The composition can also contain liquids, such as water, saline, glycerol, and ethanol, as well as substances such as wetting agents, emulsifying agents, or pH buffering agents. Liposomes,

25   such as those described in U.S. 5,422,120, WO 95/13796, WO 91/14445, or EP 524,968 B1, can also be used as a carrier for the therapeutic p40 composition.

Typically, the therapeutic *p40* composition is prepared as an injectable, either as a liquid solution or suspension; however, solid forms suitable for

30   solution in, or suspension in, liquid vehicles prior to injection can also be prepared. The composition can also be formulated into an enteric coated tablet.

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or gel capsule according to known methods in the art, such as those described in U.S. 4,853,230, EP 225,189, AU 9,224,296, and AU 9,230,801.

Proliferative disorders, such as neoplasias, dysplasias, and hyperplasias, can be treated by administration of the therapeutic *p40* composition. Neoplasias which can be treated with the therapeutic composition include, but are not limited to, melanomas, squamous cell carcinomas, and head, neck, cervix, and skin cancers. Proliferative disorders which can be treated with the therapeutic *p40* composition include disorders such as anhydric hereditary ectodermal dysplasia, congenital alveolar dysplasia, epithelial dysplasia of the cervix, fibrous dysplasia of bone, and mammary dysplasia. Hyperplasias, for example, endometrial, adrenal, breast, prostate, or thyroid hyperplasias, or pseudoepitheliomatous hyperplasia of the skin can be treated with wild-type *p40* therapeutic compositions. Even in disorders in which *p40* overexpression is not implicated, down-regulation of *p40* expression or suppression of *p40* function can have therapeutic application. In these disorders, decreasing *p40* expression or suppressing *p40* function can help to suppress tumors. Similarly, in tumors where *p40* expression is not aberrant, effecting *p40* down-regulation of *p40* expression or suppression of *p40* activity can suppress metastases.

Administration of the therapeutic agents of the invention can include local or systemic administration, including injection, oral administration, particle gun, or catheterized administration, and topical administration. Various methods can be used to administer the therapeutic *p40* composition directly to a specific site in the body. For example, a small metastatic lesion can be located and the therapeutic *p40* composition injected several times in several different locations within the body of tumor. Alternatively, arteries which serve a tumor can be identified, and the therapeutic composition injected into such an artery, in order to deliver the composition directly into the tumor. A tumor which has a necrotic center can be aspirated and the composition injected directly into the now empty center of the tumor. The therapeutic *p40* composition can be directly administered to the surface of the tumor, for example, by topical application of the composition. X-ray imaging can be used

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to assist in certain of the above delivery methods. Combination therapeutic agents, including a p40 antibody or an antisense *p40* polynucleotide and other therapeutic agents, can be administered simultaneously or sequentially.

Receptor-mediated targeted delivery of therapeutic compositions containing antisense *p40* subgenomic polynucleotides to specific tissues can also be used. Receptor-mediated DNA delivery techniques are described in, for example, Findeis et al. (1993), *Trends in Biotechnol. 11*, 202-05; Chiou et al. (1994), GENE THERAPEUTICS: METHODS AND APPLICATIONS OF DIRECT GENE TRANSFER (J.A. Wolff, ed.); Wu & Wu (1988), *J. Biol. Chem. 263*, 621-24; Wu et al. (1994), *J. Biol. Chem. 269*, 542-46; Zenke et al. (1990), *Proc. Natl. Acad. Sci. U.S.A. 87*, 3655-59; Wu et al. (1991), *J. Biol. Chem. 266*, 338-42.

Alternatively, a p40 therapeutic composition can be introduced into human cells *ex vivo*, and the cells then replaced into the human. Cells can be removed from a variety of locations including, for example, from a selected tumor or from an affected organ. In addition, the therapeutic composition can be inserted into non-tumorigenic cells, for example, dermal fibroblasts or peripheral blood leukocytes. If desired, particular fractions of cells such as a T cell subset or stem cells can also be specifically removed from the blood (*see*, for example, PCT WO 91/16116). The removed cells can then be contacted with a p40 therapeutic composition utilizing any of the above-described techniques, followed by the return of the cells to the human, preferably to or within the vicinity of a tumor. The above-described methods can additionally comprise the steps of depleting fibroblasts or other non-contaminating tumor cells subsequent to removing tumor cells from a human, and/or the step of inactivating the cells, for example, by irradiation.

Both the dose of the *p40* composition and the means of administration can be determined based on the specific qualities of the therapeutic composition, the condition, age, and weight of the patient, the progression of the disease, and other relevant factors. If the composition contains p40 antibody, effective dosages of the composition are in the range of about 5 µg to about 50 µg/kg of patient body weight, about 50 µg to about 5 mg/kg,

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about 100 μg to about 500 μg/kg of patient body weight, and about 200 to about 250 μg/kg.

Therapeutic compositions containing *p40* antisense subgenomic polynucleotides can be administered in a range of about 100 ng to about 200

5   mg of DNA for local administration in a gene therapy protocol. Concentration ranges of about 500 ng to about 50 mg, about 1 μg to about 2 mg, about 5 μg to about 500 μg, and about 20 μg to about 100 μg of DNA can also be used during a gene therapy protocol. Factors such as method of action and efficacy of transformation and expression are considerations that will effect the dosage

10   required for ultimate efficacy of the antisense *p40* subgenomic polynucleotides. Where greater expression is desired over a larger area of tissue, larger amounts of antisense *p40* subgenomic polynucleotides or the same amounts readministered in a successive protocol of administrations, or several administrations to different adjacent or close tissue portions of, for example,

15   a tumor site, may be required to effect a positive therapeutic outcome. In all cases, routine experimentation in clinical trials will determine specific ranges for optimal therapeutic effect.

The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific

20   examples which are provided herein for purposes of illustration only, and are not intended to limit the scope of the invention.

EXAMPLE 1

p40 as a tumor suppressor gene

We first examined the *p40* gene sequence using cDNA from 14 primary lung

25   cancers, and 6 head and neck squamous cell carcinoma (HNSCC) cell lines (Table 1). Two missense variants were observed. These changes involved Codon 298 (Lys to Arg) in a primary lung cancer (#7904086) and Codon 14 (Glu to Gln) in a HNSCC (#11). Although paired normal DNA was not available to confirm the somatic origin of these alterations, the conserved

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nature of these missense variants suggested that inactivation of *p40* in these human cancers is uncommon.

Because of its structural similarity to *p53*, we next examined whether *p40* demonstrated tumor suppressive effects using adenovirus vector constracts. SaOs2 cells that have no expression of *p53* (*p53 -/-*) and *p40* (Fig. 1a) were infected with a replication incompetent adenovirus containing the *p40* gene under the control of the CMV promoter and an adenovirus vector control (multiplicity of infection (MOI) =3). Cell numbers were counted on days 1, 3, 5, and 7 after infection and cells infected with the *p40* adenovirus demonstrated no difference in cell number or viability compared to the control (data not shown). In contrast, SaOs2 cells infected with a *p53* adenovirus showed rapid cell death within 48 hrs of infection. Overall, these results provided little evidence of a tumor suppressor gene function for *p40*.

p40 adenoviral vector

A full length *p40* cDNA was cloned from human prostate cDNA library as previously described[1]. The construct was then subcloned into the shuttle vector, pAdTrack-CMV. The resultant plasmid was linearized by digesting with the restriction endonuclease Pme I, and subsequently cotransformed into E. coli. BJ5183cells with an adenoviral backbone plasmid, pAdEasy-1. Recombinants were selected for kanamycin resistance, and recombination was confirmed by restriction digest analysis. The linearized recombinant plasmid was then transfected into the adenovirus packaging cell line, 293, that was described in detail previously[24].

EXAMPLE 2

Overexpression and polysomy of chromosome 3

We then examined *p40* gene expression in primary lung cancers and HNSCC cell lines by Northern analysis. We found that 10 of 14 primary lung cancers (71%) and all 6 HNSCC cell lines (100%) demonstrated *p40* gene overexpression while normal paired lung tissue and a normal embryonic lung cell line revealed virtual absence of *p40* gene expression (Fig. 1a, b). Subsequently, we examined *p40* gene amplification by fluorescent in situ

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hybridization (FISH) (Fig. 2). FISH analysis suggested 3 to 5 fold amplification of the *p40* locus in 12 of 23 primary lung cancers (52%) and all 6 HNSCC cell lines (100%) (Table 1). In some cases, a concordant increase in chromosomal arm 3q with a centromeric probe was also observed

5   confirming the presence of polysomy. The amplification data from FISH analysis correlated with expression data from Northern analysis in all 6 HNSCC cell lines and the squamous cell lung cancers (see below).

We then tested *p40* protein expression in primary lung cancers and HNSCC cell lines using immunohistochemistry. Ten of 23 primary lung cancers (43%)

10   and all 6 HNSCC cell lines (100%) had *p40* protein overexpression (Fig. 3) (Table1). As shown previously, *p40* nuclear staining was present at the basal layer of the bronchial epithelium[10]. *P40* nuclear expression was characteristic of those cancers with increased *p40* protein and, interestingly, all of the positive samples were squamous cell carcinomas and all had chromosome 3

15   polysomy or more specific *p40* amplification (Table 2). In contrast, no adenocarcinomas had *p40* protein overexpression (P<0.0001, Fisher's exact test), suggesting that low level *p40* amplification correlates with abundant *p40* protein only in squamous cell carcinoma.

We also examined the relationship between histology and *p40* gene

20   amplification in lung cancer. All 10 squamous cell carcinomas (100%) and only 2 of 13 adenocarcinomas (15%) demonstrated polysomy and/or *p40* locus amplification (p<0.0001) (Table 2). Together with our amplification and immunohistochemistry data, it appeared that true *p40* amplification was characteristic of squamous cell carcinoma of the lung and head and neck.

25   To examine a possible relationship between *p40* gene amplification and *p53* status, we proceeded with sequence analysis of the *p53* gene (exon 5 to 8) in the primary lung cancers. Remarkably, out of 10 primary lung cancers with *p40* protein overexpression, 8 (80%) had *p53* mutations (p=0.0361) (Table 2). This association suggested a possible relationship between overexpression of

30   *p40* and a tumor suppressor gene role for *p53* in human cancers.

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Table 1 *p40* status in primary lung cancers and HNSCC cell lines

Sample	sequence variants	expression (Northern)	immuno-histochemistry	increased copy number	Total
primary lung cancer					
total RNA	1/14 (7%)	10/14 (71%)			14
sections	-	-	10/23 (43%)	13/23 (57%)	23
HNSCC cell line	1/6 (17%)	6/6 (100%)	6/6 (100%)	6/6 (100%)	6

Table 2 *p40* and *p53* status in 23 primary lung cancers*

Sample	increased copy number (p40)	immuno- histochemistry (p40)	mutation (p53)
squamous cell carcinoma			
L10	+	+	+
T1	+	+	+
T3	+	+	+
T13	+	+	+
T14	+	+	-
T17	+	+	+
T20	+	+	-
T21	+	+	+
T22	+	+	+
T23	+	+	+
total	10/10 (100%)†	10/10 (100%)‡	8/10 (80%)
adenocarcinoma			
L2	+	-	-
L4	-	-	-
L6	-	-	-
L12	+	-	+
L14	-	-	-
L16	-	-	-
T24	-	-	-
T25	-	-	+
T26	-	-	+
T29	-	-	+
T31	-	-	-
T34	-	-	-
T35	-	-	-
total	2/13 (15%)	0/13 (0%)	4/13 (31%)

* Five primary lung cancers only had total RNA available for analysis
† ‡ $P < 0.0001$ (Fisher's exact test for squamous cell carcinoma vs. adenocarcinoma)

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Mutation analysis for the *p40* and *p53* gene

The PCR amplification of tumor cDNA samples consisted of 35 cycles of
95°C for 1 min, 55°C for 1min, 72°C for 1 min. The primers used were:
p40 S1 (sense), 5'-GCAGCATTGATCAATCTTACAG and *p40* AS2
(antisense), 5'-TGAATTCACGGCTCAGCTCAT; *p40* S3 (sense),
5'-CGCCATGCCTGTCTACAAAAA and *p40* AS4 (antisense),
5'-GCCTCCTAAAATGACACGTTG. (SEQ ID NOS: 5-8, respectively.)
All PCR products were purified and sequenced directly using the
AmpliCycle sequencing kit (Perkin-Elmer, Foster City, CA). The sequencing
primers were: 5'-GCCACAGTACACGAACCTGG,
5'-CGTGGTCTGTGTTATAGGGAC,
5'-TGTCCTTCCAGCAGTCGAGC, 5'-AAAAGCTGAGCACGTCACGG,
5'-CTTCACCACCTCCGTGACGT,
5'-AGGTTGGCACTGAATTCACGA,
5'-AAAATTGGACGGCGGTTCAT, 5'-GTGATGGTACGAAGCGCCC,
and ACGGGCGCTTCGTACCAT. (SEQ ID NOS: 9-17, respectively)
Mutation analysis for *p53* gene was performed as described previously[25].

Northern analysis

For primary tissues, the collected samples were grossly dissected, quickly
frozen or lysed immediately in the guanidine buffer and the RNA was
isolated using a CsCl gradient method. For cancer cell lines, total RNA was
isolated using the Trizol reagent (GIBCO BRL, Bethesda, MD). All cell
lines except FADU and SaOs2 were established in our laboratory. Northern
blot hybridization using the cDNA probes was performed as described[26].

FISH analysis

FISH was performed as previously described[27]. Specifically, 4μm thick
sections were cut out and mounted on silanized glass slides, fixed in a
methanol and glacial acetic acid (3:1) solution for 5 min and then dehydrated
in ethanol series and allowed air dry. Cell lines were fixed in the same
fixative described above and dropped onto silanized glass slides. Samples
were denatured in 70% formamide and 2xSSC at 75°C for 5 min, followed

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by dehydration in cold ethanol. The BAC probe containing *p40* was isolated as described[8] and was labeled by nick translation with digoxigenin-11-dUTP (Boehringer Mannheim, Indianapolis, IN) and the biotin-labeled centromere probe for chromosome 3 was purchased (Vysis, Downers Grove, IL). The

5   hybridization mixture consisted of 10% dextran sulfate, 50% formamide, 2xSSC, 0.1 µg of the labeled probe, 10 µg of Cot-1 DNA (GibcoBRL, Gaithersburg, MD) and 10 µg of salmon sperm DNA. Before hybridization, the mixture was denatured at 75°C for 5 min and allowed to pre-anneal at 37°C for 15 min. The probes were hybridized overnight to denatured tissue

10   sections at 37°C. After hybridization, slides were washed for 5 min with 0.5xSSC at 72°C and then incubated with rhodamine-anti-digoxigenin and FITC-avidin (Oncor, Gaithersburg, MD). The samples were counterstained with 2-phenylindole-dihydrochloride and examined under a Zeiss Axiophot epi-fluorescent microscope. Tumor areas were determined by evaluating the

15   hematoxylin and eosin stained adjacent sections. Up to 20 nuclear signals were counted under a double-band pass filter. FISH on normal specimens or non-malignant areas was analysed in the same manner as a control. For documentation, images were captured by a CCD camera (Photometrics, Tucson, AZ) and processed using the Oncor Image analysing system.

20   **Immunohistochemical analysis**

Six micron sections were made from paraffin tissue blocks and the slides were dried at 60°C for 30 min, treated with xylenes, and then dehydrated in alcohol. Endogenous peroxidase was blocked with 0.3% H2O2. After blocking with normal goat serum, the slides were incubated with the

25   polyclonal rabbit antiserum against *p40* at 1:1000 dilution for 1 hr at room temperature. *P40* antiserum was made and provided by David Hill in Oncogene Research Products (Cambridge, MA) using a *p40* specific peptide (ENNAQTQFSEPQY). A Vectastain ABC Kit and DAB Substrate Kit (Vector, Burlingame, CA) were used to visualize the antibody binding.

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Immunohistochemical analysis for *p40* was interpreted by an experienced

pathologist (W.H.W.) to determine *p40* positive and negative staining cases.

Only nuclear staining was interpreted as positive (Figure 3). For control

studies, HNSCC cell line (22) and lung cancer cell line (H1299) were used

5   as positive and negative controls. The *p40* status of these two cell lines was

confirmed by Northern analysis. Optimized conditions were then used for

the immunostaining of primary lung cancer specimens.

EXAMPLE 3

Transformation by *p40*

10   Clones that overexpress *p40* (Rat 1a-*p40*) and vector only controls

(Rat-1a-no) were generated by plasmid transfection into a parental Rat 1a

fibroblast cell line. The transformed phenotype of these cell lines was

initially assayed by culture in soft agar. Rat 1a-*p40* cells displayed a

significantly increased frequency of colony formation and larger colonies

15   compared with Rat 1a-no cells (Table 3). Pooled clones overexpressing *p40*

were inoculated into nude mice to examine the tumorigenicity of *p40 in*

vivo. Tumor size was measured at 21, 24, and 31 days after inoculation,

and we found that Rat 1a-*p40* cells produced significantly larger tumors

compared with Rat 1a-no cells (Fig. 4). These observations suggest that

20   *p40* overexpression results in a transformed phenotype and further supports

p40's role as an oncogene.

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Table 3 The number of colonies in soft agar plates from transfected Rat 1a cells

Size of colonies (μm)	200-400	400+
Rat 1a-*p40*	695±65	37±16
Rat 1a-no	363±125	9±4
p (t-test)	0.0150	0.0384

Transformation assay

A full length *p40* cDNA was cloned into pCEP4 (Invitrogen, Carlsbad, CA). pCEP4-*p40* and pCEP4 were transfected to Rat 1a fibroblast cells using Lipofectamine (GibcoBRL, Gaithersberg, MD) according to the protocol provided by the manufacturer. Cells were selected with the Hygromycin B at 200μg/ml and *P40* expression in Rat 1a-*p40* cells was confirmed by immunohistochemistry.

For soft agar analysis, 10^5 cells of either Rat 1a-*p40* or Rat 1a-no in twofold-concentrated DMEM-20% fetal bovine serum were mixed with an equal volume of 0.8% agarose and poured onto a bed of 0.7% agarose. After 18 days, colonies were counted and measured under the microscope. All experiments were performed in triplicate and differences were analysed by the t-test.

Tumor growth in nude mice was assayed by innoculating 5×10^6 cells of either Rat 1a-*p40* or Rat 1a-no into the right or left frank of 5 nude mice respectively. At 21 and 24 days after inoculation, tumor size was measured in 3 dimensions. Differences of tumor volumes were analysed by the t-test.

EXAMPLE 4

p40 displays a dominant negative effect on *p53* transcriptional activity

Based on its structural similarity, *p40* may compete for *p53* binding sites
and alter the ability of *p53* to regulate target genes. Previous reports had
suggested that ΔN*p63*, similar to *p40* in that it lacks an N-terminal domain,
could bind to *p53* target sites in a competitive manner and decrease *p53*
transactivation[10]. We thus co-infected SaOs2 cells with a constant amount
of *p53* adenovirus (MOI=2) and varying concentrations of *p40* adenovirus
(MOI=0.5 to 4), and assayed for transactivation on a consensus *p53*
promoter (PG13) driving the luciferase reporter gene[3]. As seen previously,
at an equal ratio of *p53* to *p40*, transactivation activity was reduced by
about 50% compared to *p53* alone (data not shown).

Luciferase assay

p40 and *p53* adenoviruses were co-infected into SaOs2 cells for 1 hr at
the indicated ratios. After the infection, the same amount of plasmid
containing PG13 luciferase reporter gene was transfected to these SaOs2
cells using Lipofectamine (GibcoBRL, Gaithersberg, MD) according to the
protocol provided by the manufacturer. The PG13 luciferase reporter gene
contains 13 tandem repeats of a p53 binding sequence upstream of the
luciferase reporter gene. Cells were lysed 18 hr after transfection and
luciferase activity was detected using the Luciferase Assay System
(Promega, Madison, WI). All luminescence values were measured on a
LS60001C Liquid Scintilation System (Beckman, Fullerton, CA).

EXAMPLE 5

p40 displays a dominant negative effect on *p53* transcriptional activity

Based on its structural similarity, *p40* may compete for *p53* binding sites
and alter the ability of *p53* to regulate target genes. Previous reports had
suggested that ΔN*p63*, similar to *p40* in that it lacks an N-terminal domain,
could bind to *p53* target sites in a competitive manner and decrease *p53*

35

transactivation[10]. We thus co-infected SaOs2 cells with a constant amount
of *p53* adenovirus (MOI=2) and varying concentrations of *p40* adenovirus
(MOI=0.5 to 4), and assayed for transactivation on a consensus *p53*
promoter (PG13) driving the luciferase reporter gene[3]. As seen previously,

5   at an equal ratio of *p53* to *p40*, transactivation activity was reduced by
about 50% compared to *p53* alone (data not shown).

EXAMPLE 6

p40 interacts with *p53*

       In order to further understand the function of *p40*, a yeast two-hybrid

10   screen using *p40* as a bait was undertaken. We screened 1.6×10^6 clones
from a mouse embryonic cDNA library for genes encoding proteins that
were able to bind *p40* specifically. Twenty-one clones that specifically
activated the HIS3 and LacZ reporter genes in the presence of *p40* were
analysed. After sequence analysis and database comparisons of these 21

15   clones, the most common target protein identified was *p53*, yielding 3
independent, partially overlapping clones (Fig. 5).

       The strength of this interaction is summarized in Table 4. The interaction
between 2 of the identified *p53* clones was consistent as measured by
ß-galactosidase activity and was nearly as strong as the interaction between

20   *p53* and the SV40 positive control. Examination of the region of overlap
within the clones identified the DNA binding domain as the probable
interaction motif.

Table 4 The strength of the interaction between bait and prey

Bait	Prey	Trp+	Leu+	His+	β-galactosidase (nmole/min/mg)
pGal4-BD-p53	pGal4-AD-SV-40	+	+	+	65.3±6.3
pGal4-BD-laminC	pGal4-AD-SV-40	+	+	-	2.6±0.9
pGal4-p40		+	-	-	3.7±1.2
pGal4-p40	pGal4-AD	+	+	-	4.2±1.1
pGal4-AD-p53-1		-	+	-	3.3±0.8
pGal4-AD-p53-2		-	+	-	3.4±0.5
pGal4-BD-p40	pGal4-AD-p53-1	+	+	+	33.6±3.7
pGal4-BD-p40	pGal4-AD-p53-2	+	+	+	31.8±2.9

Yeast two-hybrid screen

To screen for potential binding partners of *p40*, we employed a yeast two-hybrid system and screened the Hybri-Zap-Gal4 mouse embryonic cDNA library (#977317, mouse strain B6:C57BL/6, day 14.5, Stratagene, LaJolla, CA). To prepare the bait plasmid, human *p40* cDNA was inserted 3' to the cDNA for the Gal4 binding domain of plasmid pGal4-BD (Stratagene, CA). The resultant pGal4-BD-p40 bait plasmid encoded a fusion protein comprised of the Gal4 binding domain and a *p40* protein. YRG-2 yeast cells were co-transformed with the pGal4-BD-p40 bait plasmid and a Hybri-Zap-Gal4-based library, and then grown on a selective medium lacking tryptophan, leucin and histidine or combinations thereof. Colonies that contained a cDNA encoding target library proteins interacting with the bait fusion protein were identified by the assay based on activation of transcription of the yeast chromosomal HIS3- and lacZ-genes (filter-lift assay). A total of 1.6×10^6 yeast transformants were placed under Trp-Leu-His- selection. Plasmid preparations from β-gal positive yeast colonies were isolated and re-transformed into library-efficiency competent E. coli DH5α cells.

The ampicillin resistant colonies were then grown up and DNA preps were analysed by EcoRI/XhoI restriction mapping followed by DNA

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sequencing. To ensure that cDNA clones obtained from the first screen encoded true positive *p40*-interactors we introduced pGal4-BD-p40 and pGal4-AD-clones into *Saccharomyces cerevisiae* (strain SFY 526) together and separately, as well as with positive and negative control pairs

5   (pGal4-BD-p53 plus pGal4-AD-SV-40, and pGal4-BD-lamin C plus pGal4-AD-SV-40, respectively). Yeast colonies were grown up in agar-agar plates lacking tryptophan, leucin or tryptophan plus leucine and then growing colonies were transferred into agar-agar plates lacking tryptophan, leucine and histidine. Yeast colonies that were grown on latter

10   plates were subjected to quantitative liquid β-galactosidase activity assay using o-nitrophenyl-β-D-galactopyranoside (OPNG) as a substrate (nmol OPNG cleaved/min/mg protein measured at OD420)[28].

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5

10

CLAIMS

1.   An isolated and purified p40 protein having an amino acid sequence which is at least 99% identical to SEQ ID NO:2, wherein percent identity is determined using a Smith-Waterman homology

5   search algorithm using an affine gap search with a gap open penalty of 12 and a gap extension penalty of 1.

2.   The isolated and purified p40 protein of claim 1 which has the amino acid sequence shown in SEQ ID NO:2.

3.   A p40 fusion protein comprising a first protein segment

10   and a second protein segment fused together by means of a peptide bond, wherein the first protein segment consists of a p40 protein as shown in SEQ ID NO:2.

4.   A preparation of antibodies which specifically bind to a p40 protein having an amino acid sequence as shown in SEQ ID NO:2,

15   but which does not bind to p53 as shown in SEQ ID NO: 4.

5.   A cDNA molecule which encodes a p40 protein having an amino acid sequence which is at least 99% identical to SEQ ID NO:2, wherein percent identity is determined using a Smith-Waterman homology search algorithm using an affine gap search with a gap open

20   penalty of 12 and a gap extension penalty of 1.

6.   The cDNA molecule of claim 5 which comprises a nucleotide sequence shown in SEQ ID NO:1.

7.   A cDNA molecule which is at least 99% identical to the nucleotide sequence shown in SEQ ID NO:1, wherein percent identity

25   is determined using a Smith-Waterman homology search algorithm using an affine gap search with a gap open penalty of 12 and a gap extension penalty of 1.

8.   A nucleic acid construct comprising:
       a promoter; and

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a polynucleotide segment encoding a p40 protein as shown in SEQ ID NO:2, wherein the polynucleotide segment is located downstream from the promoter, wherein transcription of the polynucleotide segment initiates at the promoter, wherein the promoter is not the endogenous p40 promoter.

5

9. A host cell comprising a nucleic acid construct which comprises:

a promoter and:

a polynucleotide segment encoding a p40 protein having an amino acid sequence as shown in SEQ ID NO:2, wherein the promoter is not the endogenous p40 promoter.

10

10. A method of diagnosing a neoplastic tissue of a human, comprising the step of:

detecting amplification of a *p40* gene in a tissue suspected of being neoplastic, wherein the *p40* gene has the coding sequence shown in SEQ ID NO:1, wherein the amplification indicates neoplasia of the tissue.

15

11. The method of claim 10 wherein the tissue suspected of being neoplastic is selected from the group consisting of head, neck, cervix, lung, and skin.

20

12. The method of claim 10 wherein the tissue suspected of being neoplastic comprises squamous cells.

13. A method of identifying or classifying a neoplastic tissue of a human, comprising the step of:

25

comparing expression of a first *p40* gene in a first tissue of a human suspected of being neoplastic with expression of a second *p40* gene in a second tissue of the human which is normal, wherein the second *p40* gene has the coding sequence shown in SEQ ID NO:1, wherein increased expression of the first *p40* gene relative to the second *p40* gene identifies the first tissue as being neoplastic and having a *p40* amplification.

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14.   The method of claim 13 wherein the expression is compared by comparing p40 proteins.

15.   The method of claim 13 wherein expression is compared by comparing p40 mRNA.

16.   The method of claim 13 wherein the tissue suspected of being neoplastic is selected from the group consisting of head, neck, cervix, lung, and skin.

17.   The method of claim 13 wherein the tissue suspected of being neoplastic comprises squamous cells.

18.   A method of screening test compounds for the ability to modulate the binding of a p40 protein to a p53 protein, comprising the steps of:

(a) contacting a test compound with a first protein comprising a p53 protein as shown in SEQ ID NO:4 and a second protein comprising a p40 protein as shown in SEQ ID NO:2, wherein the first and second proteins bind to each other in the absence of the test compound; and

(b) determining the amount of the first protein which is bound or unbound to the second protein or determining the amount of the second protein which is bound or unbound to the first protein in the presence of the test compound, wherein a test compound which modulates the amount of bound first or second protein or which modulates the amount of unbound first or second protein is a potential drug for treating cancer.

19.   The method of claim 18 wherein the first and second proteins are prebound prior to the step of contacting.

20.   The method of claim 18 wherein the test compound is contacted with either of the first or second protein prior to the step of contacting.

21.   The method of claim 18 wherein the p53 protein is wild-type p53.

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22.   The method of claim 18 wherein the p53 protein is a mutant p53.

23.   The method of claim 18 wherein the first and second proteins are expressed in a cell and the test compound is contacted with the cell.

24.   The method of claim 23 wherein bound first or second protein is determined by assaying expression of a gene which is transcriptionally activated by p53, wherein a test compound which decreases expression of the gene which is transcriptionally activated by p53 increases the amount of bound first or second protein.

25.   A method of screening test compounds for the ability to modulate the binding of a p53 protein to a p40 protein, comprising the steps of:

(a) contacting a cell with a test compound, wherein the cell comprises:

i) a first fusion protein comprising (1) a p40 protein as shown in SEQ ID NO:2 and (2) either a DNA binding domain or a transcriptional activating domain;

ii) a second fusion protein comprising a p53 protein as shown in SEQ ID NO:4 and (2) either a DNA binding domain or a transcriptional activating domain, wherein if the first fusion protein comprises a DNA binding domain, then the second fusion protein comprises a transcriptional activating domain, wherein if the first fusion protein comprises a transcriptional activating domain, then the second fusion protein comprises a DNA binding domain, wherein the interaction of the first and second fusion proteins reconstitutes a sequence-specific transcription activating factor; and

iii) a reporter gene comprising a DNA sequence to which the DNA binding domain specifically binds; and

(b) measuring the expression of the reporter gene, wherein a test compound which modulates the expression of the reporter gene is a potential anti-cancer drug.

26. A cell which comprises three recombinant DNA constructs, wherein a first construct encodes a first polypeptide fused to a sequence-specific DNA-binding domain, wherein a second construct encodes a second polypeptide fused to a transcriptional activation domain, and wherein a third construct comprises a reporter gene downstream from a DNA element which is recognized by the sequence-specific DNA-binding domain, wherein the first polypeptide comprises a p40 protein as shown in SEQ ID NO:2 and the second polypeptide comprises a p53 protein as shown in SEQ ID NO:4, or the first polypeptide comprises a p53 protein as shown in SEQ ID NO:4 and the second polypeptide comprises a p40 protein as shown in SEQ ID NO:2.

27. A method of visualizing a human chromosomal arm 3q, comprising the steps of:

contacting a preparation of metaphase human chromosomes with a nucleotide probe comprising at least 12 contiguous nucleotides selected from the nucleotide sequence shown in SEQ ID NO:1; and

detecting a chromosome which specifically hybridizes to the nucleotide probe, wherein a chromosome which specifically hybridizes to the nucleotide probe is identified as a human chromosomal arm 3q.

28. The method of claim 27 wherein the nucleotide probe is fluorescently labeled.

29. The method of claim 27 further comprising the step of quantitating the amount of the nucleotide probe which hybridizes to chromosomal arm 3q.

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30.   A therapeutic composition for treating neoplasia,
comprising:

a therapeutically effective amount of an antisense *p40*
polynucleotide; and

5   a pharmaceutically acceptable carrier.

31.   A therapeutic composition comprising:

a therapeutically effective amount of an antibody which
specifically binds to a human p40 protein; and

a pharmaceutically acceptable carrier.

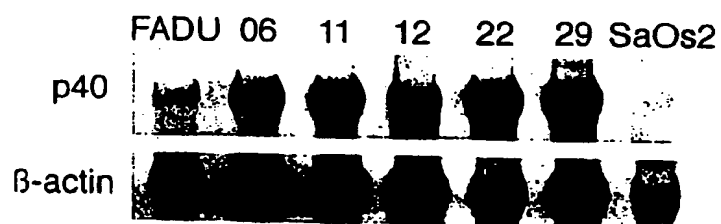10   32.   A method of treating neoplasia, comprising the step of:

administering to a patient with neoplasia a therapeutically

effective amount of a therapeutic *p40* composition, whereby the

patient's neoplasia is reduced.

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Fig 1

A

B

Fig 2 or 3

Fig 4

Fig 5

TRP-/LEU-/HIS-

p40+p53-1   p40+p53-2   p53+SV40

p40+pAD►   ◄p53-1

p40◄   ►p53-2

lamin C+SV40

Fig 4

Fig 7

A. Blot: anti-p53 anti-MK

200 →

94 →
66 → ← GST-p53

43 → ← GST-MK

31 →

B.

1 2 3 4

← p40
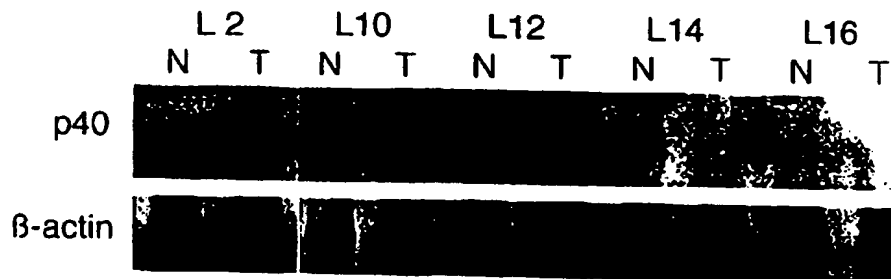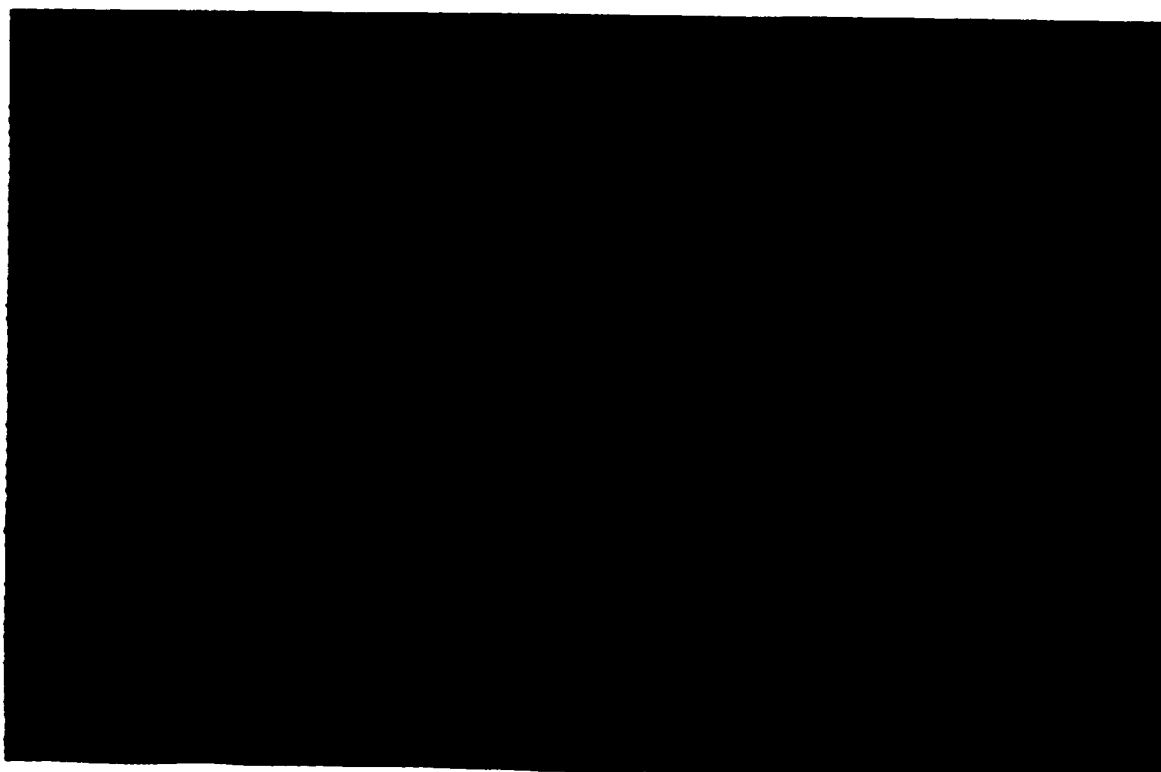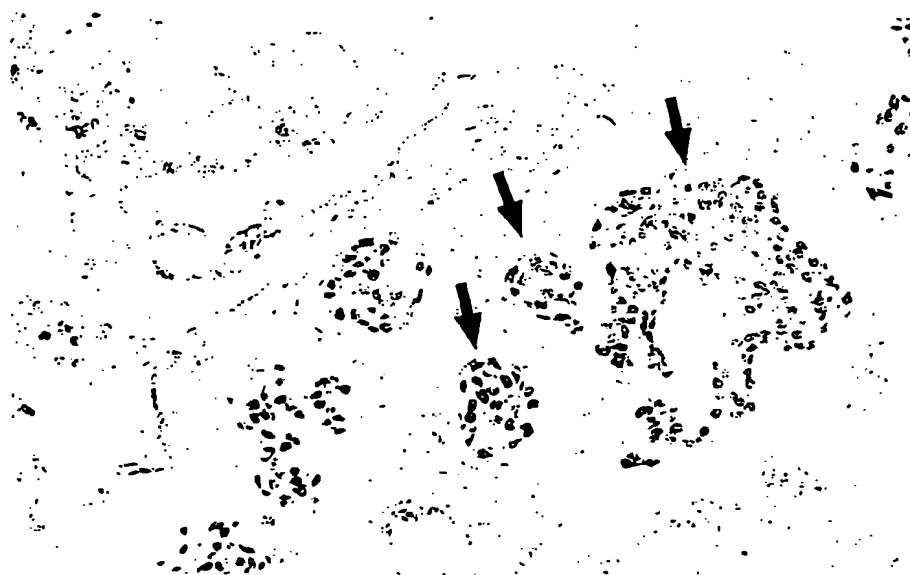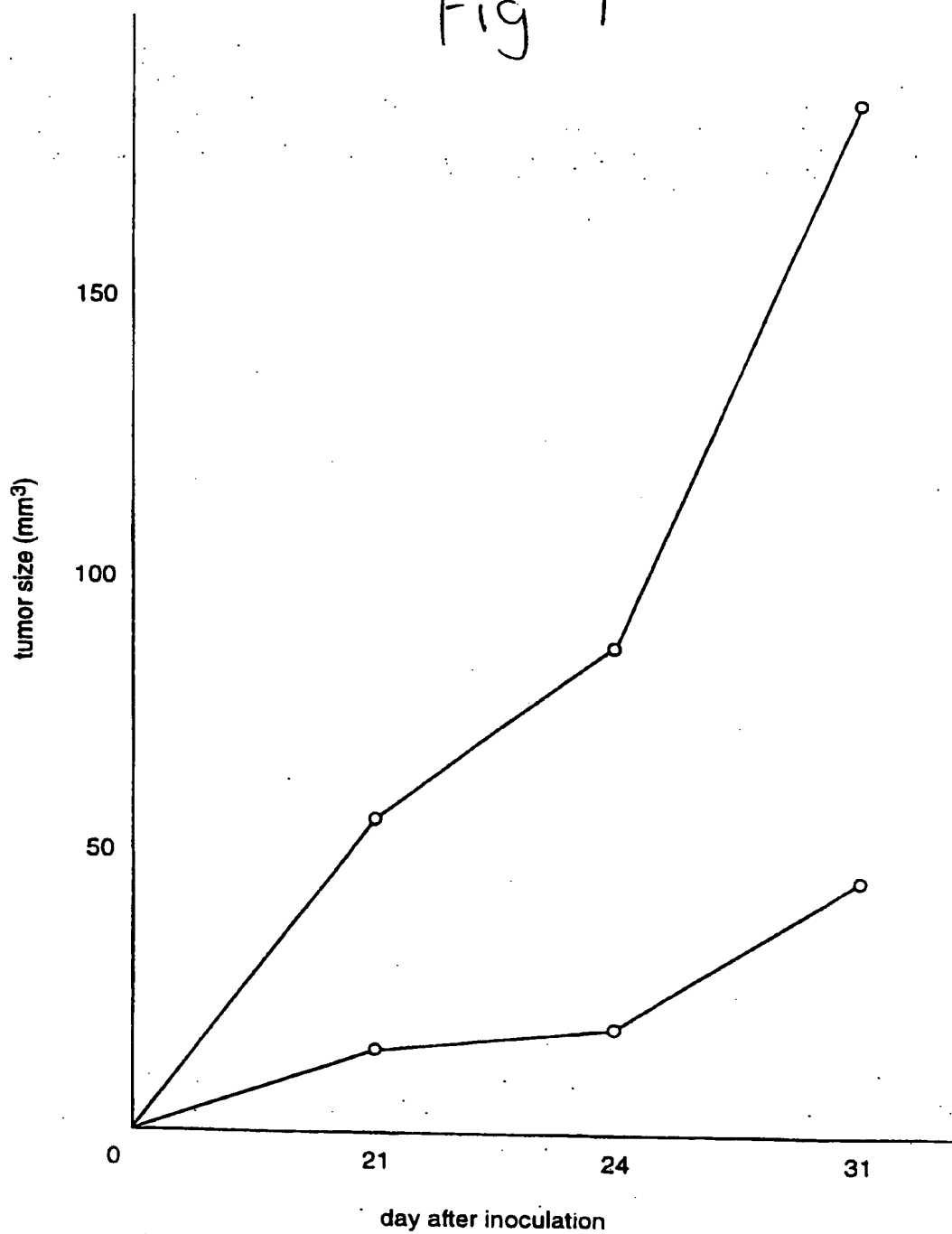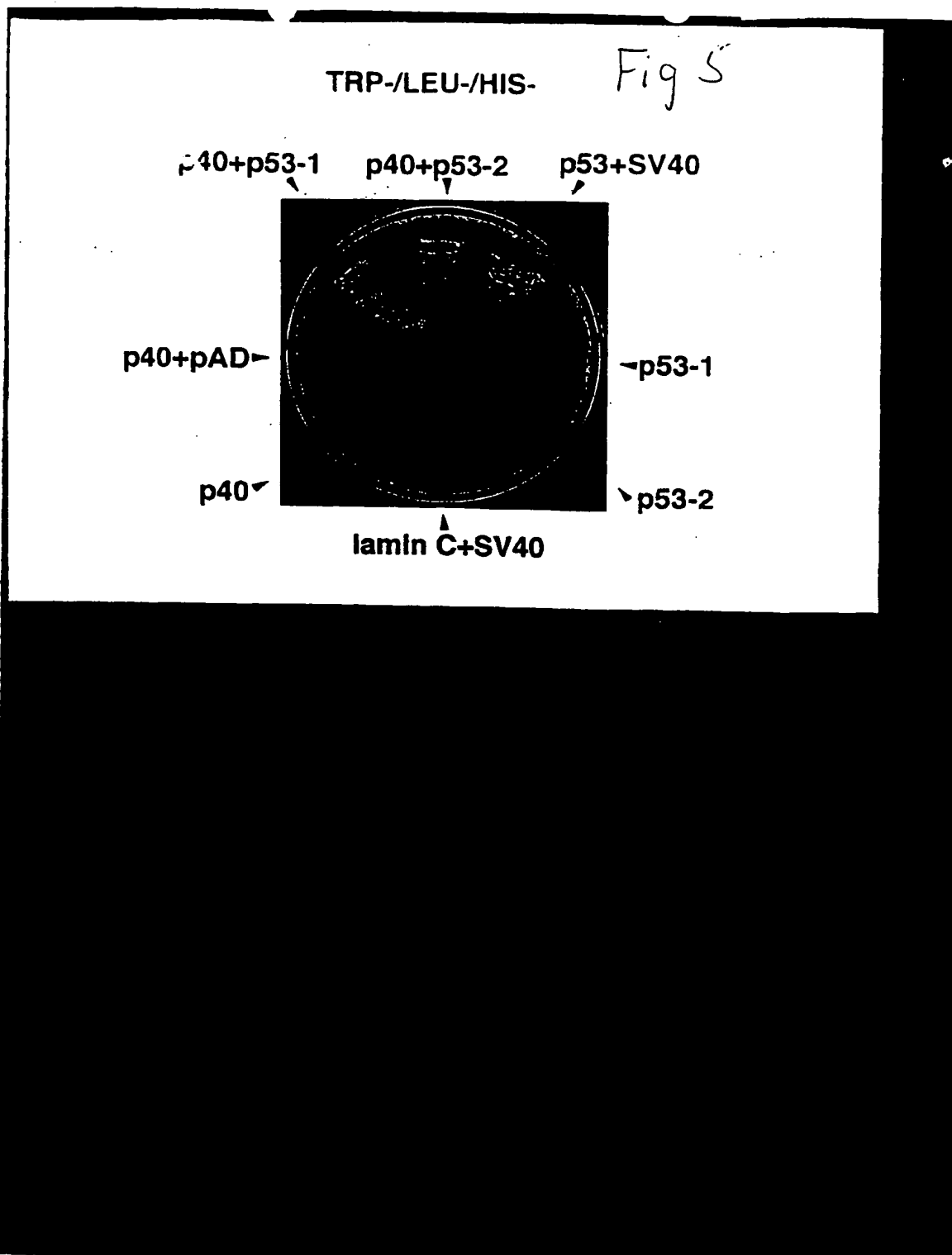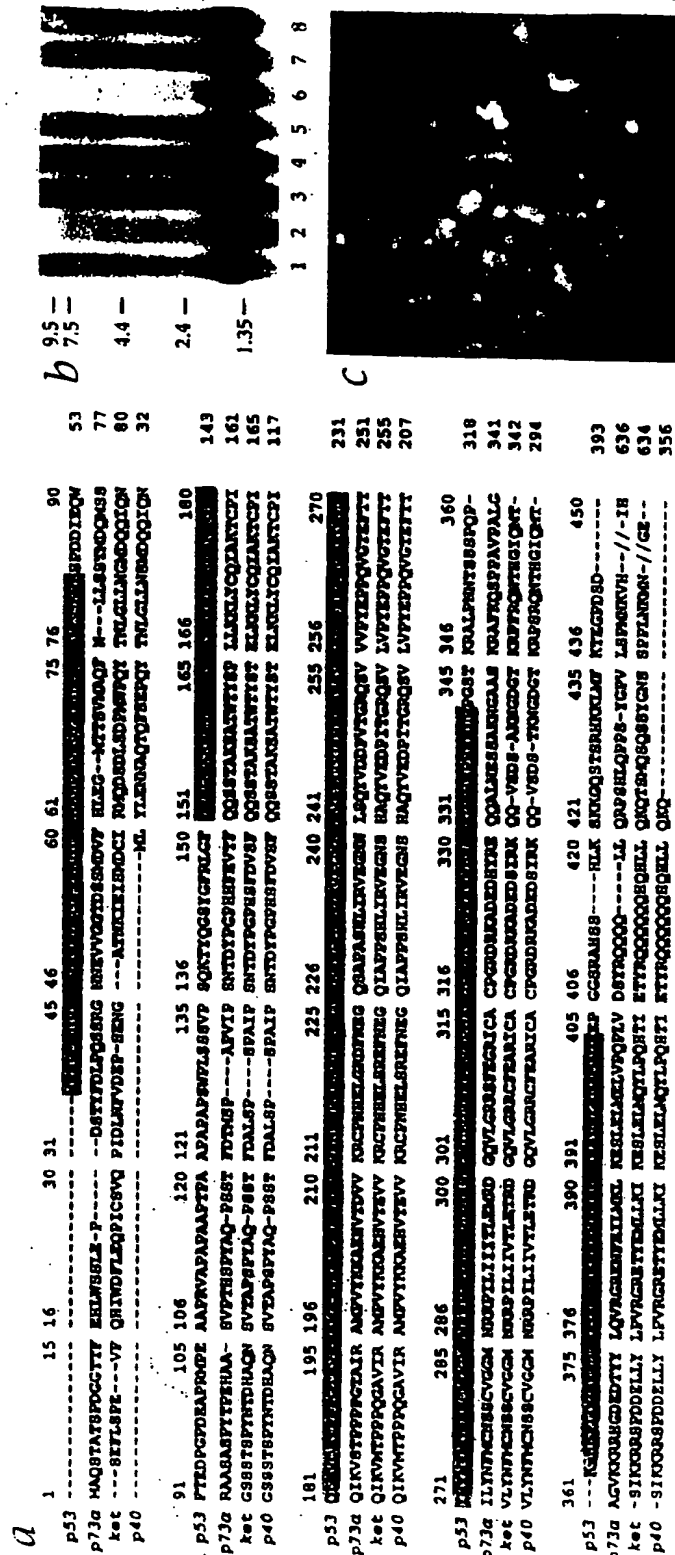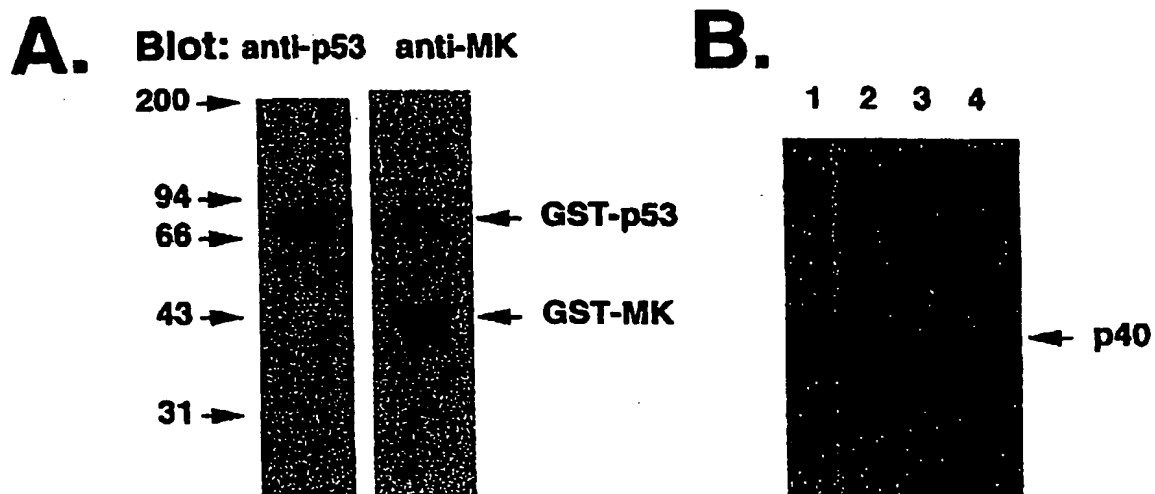
Fig 8

A. Blot: anti-p40

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66 →

43 →

31 →
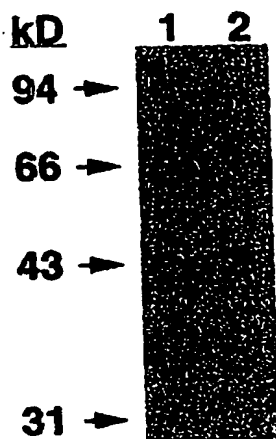
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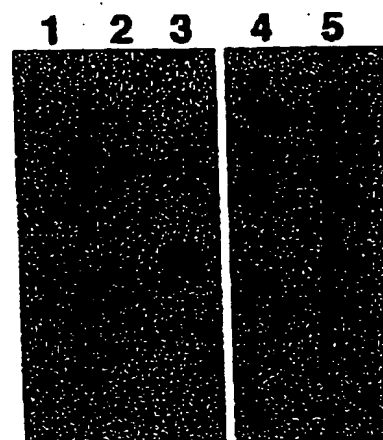
IP: glutathione agarose   anti-p40

Fig 9

SEQUENCE LISTING

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        <400> 2
Met Leu Tyr Leu Glu Asn Asn Ala Gln Thr Gln Phe Ser Glu Pro Gln
1               5                   10                  15
Tyr Thr Asn Leu Gly Leu Leu Asn Ser Met Asp Gln Gln Ile Gln Asn
            20                  25                  30
Gly Ser Ser Ser Thr Ser Pro Tyr Asn Thr Asp His Ala Gln Asn Ser
        35                  40                  45
Val Thr Ala Pro Ser Pro Tyr Ala Gln Pro Ser Ser Thr Phe Asp Ala
    50                  55                  60
Leu Ser Pro Ser Pro Ala Ile Pro Ser Asn Thr Asp Tyr Pro Gly Pro
65                  70                  75                  80
His Ser Phe Asp Val Ser Phe Gln Gln Ser Ser Thr Ala Lys Ser Ala
                85                  90                  95
Thr Trp Thr Tyr Ser Thr Glu Leu Lys Lys Leu Tyr Cys Gln Ile Ala
                100                 105                 110
Lys Thr Cys Pro Ile Gln Ile Lys Val Met Thr Pro Pro Pro Gln Gly
        115                 120                 125
Ala Val Ile Arg Ala Met Pro Val Tyr Lys Lys Ala Glu His Val Thr
    130                 135                 140
Glu Val Val Lys Arg Cys Pro Asn His Glu Leu Ser Arg Glu Phe Asn
145                 150                 155                 160
Glu Gly Gln Ile Ala Pro Pro Ser His Leu Ile Arg Val Glu Gly Asn
                165                 170                 175
Ser His Ala Gln Tyr Val Glu Asp Pro Ile Thr Gly Arg Gln Ser Val
            180                 185                 190
Leu Val Pro Tyr Glu Pro Pro Gln Val Gly Thr Glu Phe Thr Thr Val
        195                 200                 205
Leu Tyr Asn Phe Met Cys Asn Ser Ser Cys Val Gly Gly Met Asn Arg
    210                 215                 220
Arg Pro Ile Leu Ile Ile Val Thr Leu Glu Thr Arg Asp Gly Gln Val
225                 230                 235                 240
Leu Gly Arg Arg Cys Phe Glu Ala Arg Ile Cys Ala Cys Pro Gly Arg
                245                 250                 255
Asp Arg Lys Ala Asp Glu Asp Ser Ile Arg Lys Gln Gln Val Ser Asp
            260                 265                 270
Ser Thr Lys Asn Gly Asp Gly Thr Lys Arg Pro Ser Arg Gln Asn Thr
        275                 280                 285
His Gly Ile Gln Met Thr Ser Ile Lys Lys Arg Arg Ser Pro Asp Asp
    290                 295                 300
Glu Leu Leu Tyr Leu Pro Val Arg Gly Arg Glu Thr Tyr Glu Met Leu
305                 310                 315                 320
Leu Lys Ile Lys Glu Ser Leu Glu Leu Met Gln Tyr Leu Pro Gln His
            325                 330                 335
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2

Thr Ile Glu Thr Tyr Arg Gln Gln Gln Gln Gln Gln His Gln His Leu
            340   345   350
Leu Gln Lys Gln
        355

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        <210> 3
        <211> 2065
        <212> DNA
        <213> Homo sapiens

        <400> 3
caaaacctac cagggcagct accgtttccg tctgggcttc ttgcattctg ggacagccaa     60
gtctgtgact tgcacgtact cccctgccct caacaagatg ttttgccaac tggccaagac    120
ctgccctgtg cagctgtggg ttgattccac accccgccc ggcacccgcg tccgcgccat    180
ggccatctac aagcagtcac agcacatgac ggaggttgtg aggcgctgcc cccaccatga    240
gcgctgctca gatagcgatg gtctggcccc tcctcagcat cttatccgag tggaaggaaa    300
tttgcgtgtg gagtatttgg atgacagaaa cacttttcga catagtgtgg tggtgcccta    360
tgagccgcct gaggaggttg gctctgactg taccaccatc cactacaact acatgtgtaa    420
cagttcctgc atgggcggca tgaaccggag gcccatcctc accatcatca cactggaaga    480
ctccagtggt aatctactgg gacggaacag ctttgaggtg cgtgtttgtg cctgtcctgg    540
gagagaccgg cgcacagagg aagagaatct ccgcaagaaa ggggagcctc accacgagct    600
gcccccaggg agcactaagc gagcactgcc caacaacacc agctcctctc cccagccaaa    660
gaagaaacca ctggatggag aatatttcac ccttcagatc cgtgggcgtg agcgcttcga    720
gatgttccga gagctgaatg aggccttgga actcaaggat gcccaggctg ggaaggagcc    780
agggggggagc agggctcact ccagccacct gaagtccaaa aaggctcagt ctacctcccg    840
ccataaaaaa ctcatgttca agacagaagg gcctgactca gactgacatt ctccacttct    900
tgttccccac tgacagcctc ccaccccat ctctccctcc cctgccattt tgggttttgg    960
gtctttgaac ccttgcttgc aataggtgtg cgtcagaagc acccaggact tccatttgct   1020
ttgtcccggg gctccactga acaagttggc ctgcactgct gttttgttgt ggggaggagg   1080
atggggagta ggacatacca gcttagattt taaggttttt actgtgaggg atgtttggga   1140
gatgtaagaa atgttcttgc agttaagggt tagtttacaa tcagccacat tctaggtagg   1200
ggcccacttc accgtactaa ccagggaagc tgtccctcac tgttgaattt tctctaactt   1260
caaggcccat atctgtgaaa tgctggcatt tgcacctacc tcacagagtg cattgtgagg   1320
gttaatgaaa taatgtacat ctggccttga aaccacctt tattacatgg ggtctagatg   1380
accccctgga ggtgcttgtt ccctctccct gttggtcggt gggttggtag tttctacagt   1440
tgggcagctg gttaggtaga gggagttgtc aagtctctgc tggcccagcc aaaccctgtc   1500
tgacaacctc ttggtgaacc ttagatccta aaaggaaatg tcaccccatc ccacaccctg   1560
gaggatttca tctcttgtat agatgatctg gatccaccaa gacttgttgt tttagctcag   1620
ggtccaattt ctttttttctt ttttttttttt ttttttcttttt tctttgagac tgggtctctt   1680
tgttgcccca ggctggagtg gagtggcgtg atctggctta ctgcagcctt tgcctccccg   1740
gctcgagcag tcctgcctca gcctccggag tagctgggac cacaggttca tgccaccatg   1800
gccagccaac ttttgcatgt tttgtagaga tggggtctca cagtgttgcc caggctggtc   1860
tcaaactcct gggctcaggc gatccacctg tctcagcctc ccagagtggg attacaattg   1920
tgagccacca cgtccagctg gaagggtcaa catcttttac attctgcaag cacatctgca   1980
ttttcacccc accctteccc tcttctccct ttttatatcc cattttttata tcgatctctt   2040
attttacaat aaaacttgc tgcca                                          2065

        <210> 4
        <211> 393
        <212> PRT
        <213> Homo sapiens

        <400> 4
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Met Glu Glu Pro Gln Ser Asp Pro Ser Val Glu Pro Pro Leu Ser Gln
1   5   10   15

3

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Glu Thr Phe Ser Asp Leu Trp Lys Leu Leu Pro Glu Asn Asn Val Leu
         20                  25              30
Ser Pro Leu Pro Ser Gln Ala Met Asp Asp Leu Met Leu Ser Pro Asp
         35                  40              45
Asp Ile Glu Gln Trp Phe Thr Glu Asp Pro Gly Pro Asp Glu Ala Pro
    50                  55              60
Arg Met Pro Glu Ala Ala Pro Arg Val Ala Pro Ala Pro Ala Ala Pro
65                  70                  75                  80
Thr Pro Ala Ala Pro Ala Pro Ala Pro Ser Trp Pro Leu Ser Ser Ser
             85                  90                  95
Val Pro Ser Gln Lys Thr Tyr Gln Gly Ser Tyr Gly Phe Arg Leu Gly
         100                 105             110
Phe Leu His Ser Gly Thr Ala Lys Ser Val Thr Cys Thr Tyr Ser Pro
         115                 120             125
Ala Leu Asn Lys Met Phe Cys Gln Leu Ala Lys Thr Cys Pro Val Gln
    130                 135             140
Leu Trp Val Asp Ser Thr Pro Pro Pro Gly Thr Arg Val Arg Ala Met
145                 150                 155                 160
Ala Ile Tyr Lys Gln Ser Gln His Met Thr Glu Val Val Arg Arg Cys
             165                 170                 175
Pro His His Glu Arg Cys Ser Asp Ser Asp Gly Leu Ala Pro Pro Gln
         180                 185             190
His Leu Ile Arg Val Glu Gly Asn Leu Arg Val Glu Tyr Leu Asp Asp
         195                 200             205
Arg Asn Thr Phe Arg His Ser Val Val Val Pro Tyr Glu Pro Pro Glu
    210             215             220
Val Gly Ser Asp Cys Thr Thr Ile His Tyr Asn Tyr Met Cys Asn Ser
225                 230                 235                 240
Ser Cys Met Gly Gly Met Asn Arg Arg Pro Ile Leu Thr Ile Ile Thr
             245                 250             255
Leu Glu Asp Ser Ser Gly Asn Leu Leu Gly Arg Asn Ser Phe Glu Val
         260                 265             270
Arg Val Cys Ala Cys Pro Gly Arg Asp Arg Arg Thr Glu Glu Glu Asn
    275             280             285
Leu Arg Lys Lys Gly Glu Pro His His Glu Leu Pro Pro Gly Ser Thr
    290             295             300
Lys Arg Ala Leu Pro Asn Asn Thr Ser Ser Ser Pro Gln Pro Lys Lys
305             310             315                 320
Lys Pro Leu Asp Gly Glu Tyr Phe Thr Leu Gln Ile Arg Gly Arg Glu
             325             330             335
Arg Phe Glu Met Phe Arg Glu Leu Asn Glu Ala Leu Glu Leu Lys Asp
             340             345             350
Ala Gln Ala Gly Lys Glu Pro Gly Gly Ser Arg Ala His Ser Ser His
         355             360             365
Leu Lys Ser Lys Lys Gly Gln Ser Thr Ser Arg His Lys Lys Leu Met
    370             375             380
Phe Lys Thr Glu Gly Pro Asp Ser Asp
385             390
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    <210> 5
    <211> 22
    <212> DNA
    <213> Homo sapiens

    <400> 5
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gcagcattga tcaatcttac ag   22

    <210> 6
    <211> 21
    <212> DNA
    <213> Homo sapiens

    <400> 6
tgaattcacg gctcagctca t   21

    <210> 7
    <211> 21
    <212> DNA
    <213> Homo sapiens

    <400> 7
cgccatgcct gtctacaaaa a   21

    <210> 8
    <211> 21
    <212> DNA
    <213> Homo sapiens

    <400> 8
gcctcctaaa atgacacgtt g   21

    <210> 9
    <211> 20
    <212> DNA
    <213> Homo sapiens

    <400> 9
gccacagtac acgaacctgg   20

    <210> 10
    <211> 21
    <212> DNA
    <213> Homo sapiens

    <400> 10
cgtggtctgt gttataggga c   21

    <210> 11
    <211> 20
    <212> DNA
    <213> Homo sapiens

    <400> 11
tgtccttcca gcagtcgagc   20

    <210> 12
    <211> 20
    <212> DNA
    <213> Homo sapiens

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        <400> 12
aaaagctgag cacgtcacgg                                               20


        <210> 13
        <211> 20
        <212> DNA
        <213> Homo sapiens


        <400> 13
cttcaccacc tccgtgacgt                                               20


        <210> 14
        <211> 21
        <212> DNA
        <213> Homo sapiens


        <400> 14
aggttggcac tgaattcacg a                                             21


        <210> 15
        <211> 20
        <212> DNA
        <213> Homo sapiens


        <400> 15
aaaattggac ggcggttcat                                               20


        <210> 16
        <211> 19
        <212> DNA
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        <400> 16
gtgatggtac gaagcgccc                                                19


        <210> 17
        <211> 18
        <212> DNA
        <213> Homo sapiens


        <400> 17
acgggcgctt cgtaccat                                                 18


        <210> 18
        <211> 13
        <212> PRT
        <213> Homo sapiens


        <400> 18
Glu Asn Asn Ala Gln Thr Gln Phe Ser Glu Pro Gln Tyr
1               5               10
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6